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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)

T helper, CD4+lymphocytes, recover slowly following chemotherapy. In healthy females, exercise increased the circulating levels of CD4+cells. Our goal was to determine if exercise would help CD4+ cell recovery following chemotherapy in breast cancer patients. Blood lymphocytes, before and after chemotherapy/radiation and 3 and 6 months of exercise were assayed for proliferation and for expression of leukocyte antigens. Following fitness testing, the subjects exercised with a trainer. Questionnaires (quality of life, diet, and physical activity) were administered 5 times. Twenty-eight women completed at least 3 mo of exercise, 21 completed 6 mo, and 21 women were in the control (no formal exercise) group. There was a significant ($p<0.05$) increase in quality of life, upper body strength, and cardiovascular function in the exercise group. These changes were not seen in the control group.The total CD4+ T lymphocytes did not reach the normal range for either group; however, the exercisers showed a significant increase in the total number of naïve (CD45RA) cells of the CD4+ lineage. This novel finding suggests that exercise may activate thymic function which results in production of more CD4+ T lymphocytes. In summary, exercise resulted in predicted positive physical and psychological changes but also led to improvements in the cells of the immune system.

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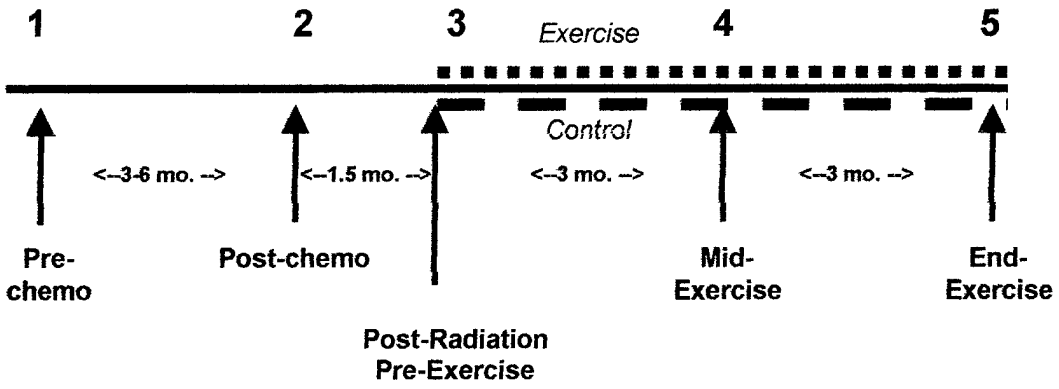
INTRODUCTION

Breast cancer is estimated to affect one in eight women in her lifetime. Treatment usually includes chemotherapy which can lead to several serious physical, emotional, and functional side effects. Along with the tumor cells, the normal, continually renewing cells of the lymphatic/immune hematopoietic system are targets for the cytotoxic drugs. One population of lymphocytes, CD4+T cells (T helper cells), major regulators of the immune system, are particularly susceptible to chemotherapy-induced depletion. CD4+T cells levels in the blood frequently fall to those seen in AIDS patients (Kilmas et al., 1991) and remain low for many months to several years after chemotherapy ceases (Greenberg & Riddell, 1999; Hakim et al., 1997). An increase in CD4+ T cells has been measured in normal, healthy females after 3 months of resistance exercise training. In addition, studies have suggested a link between continued exercise participation and improved immune parameters in AIDS patients. Increases in CD4+T cells in HIV seropositive individuals following exercise training have been reported (LaPierrere et al, 1997). No further decline or a slight increase (MacArthur, et al, 1993) was noted in those with CD4+ counts of approximately 200 cells/mm3. However, in cancer patients who have undergone chemotherapy treatment and have CD4+ counts similar to those in AIDS patients, there are no studies relating exercise and immune parameters. In the human studies the relationship between physical activity and the risk for developing cancer have been examined. The few exercise interventions documented in the literature have focused on outcomes such as fatigue (Dimeo, et al., 1997), physical function (Dimeo, et al, 1998; Mock, et al., 1997; Segar, et al., 1998) or quality of life (Courneya & Friedenreich , 1999). In addition, these exercise studies have been conducted either in populations who are currently hospitalized for stem cell transplantation or in populations who are many months beyond their completion of treatment. Based on preliminary evidence that a strength training program may enhance CD4+ cell counts in a college-aged population (Mastro et al., unpublished) and that chemotherapy treatment results in declines of this immune parameter, we hypothesized that an appropriately designed exercise training program would help in the recovery of CD4+ T lymphocyte levels following chemotherapy.

BODY

STUDY DESIGN

A timeline of the potential events during the study for an individual included five possible data collection points following diagnosis of breast cancer and lumpectomy or mastectomy.



1. Time 1 (T1)- Pre-chemotherapy blood draw and completion of questionnaires
2. Participant undergoes chemotherapy (3- 6 months dependent upon therapy type)
3. Time 2 (T2)- Pre-radiation therapy/Post-chemotherapy blood draw and completion of questionnaires
4. Participant undergoes 4-7 weeks of radiation therapy (71% of the women had radiation therapy)
6. Time 3 (T3)- Post-Therapy Baseline. Pre-exercise evaluation occurred after completion of radiation therapy (fitness testing, blood draw, and completion of questionnaires)
7. Participant begins exercise training as soon after fitness testing as possible
7. Time 4 (T4) – Mid-Study/ Mid-Exercise. Participant finishes 3 months of exercise and undergoes fitness testing, blood draw, and completion of questionnaires
8. Time 5 (T5) – End-Study/ End-Exercise Participant finishes 6 months of exercise an undergoes fitness testing, blood draw, and completion of questionnaires

Self-Report Measures

Three questionnaires were distributed to subjects at each time point of their participation, usually by mail or at a testing session. Return postpaid envelopes were provided. If questionnaires were not returned, follow-up phone calls were made to assure receipt, and encourage completion and return. Second sets of questionnaires were mailed if necessary.

Quality of Life was assessed using the Functional Assessment of Cancer Therapy (FACT)- AN (Yellen, et al, 1997) This questionnaire was developed specifically for cancer research and has the following subscales: physical well-being, social/family well-being, emotional well-being, functional well-being, and fatigue. On a 5 point scale, respondents indicated their agreement with statements about their current quality of life (0= not at all, 4= very much). Validity and reliabilty have been well-established.

Physical activity was measured using the Paffenbarger Physical Activity Questionnaire (Paffenbarger et al, 1978) and kilocalories expended weekly were calculated by the method

suggested by Paffenbarger. The calculation takes into account the energy exerted in climbing flights of stairs, walking city blocks and participating in sports and leisure activities. It was originally developed for a study of Harvard alumni and it successfully predicted incidence of coronary heart disease over a 12 – 16 year period of follow-up. It has been used in a variety of studies including studies of physical activity in women in which reliability was demonstrated. (Cauley et al, 1987; Laporte et al, 1983).

Diet information was assessed using the Client Food Questionnaire, a food frequency form adapted from the Nutritionist Five Program, First DataBank of San Bruno, California based on work by Block (Block, et al, 1986; Block, et al, 1989). It was used to record all of the food, beverages, supplements consumed in a week.

Blood Assays (see ATTACHMENT #1)

Whole Blood Mitogen Assays

Whole blood from each individual was cultured with three activators over a range of concentrations to determine the the degree of lymphocyte division in culture. The three polyclonal mitogens and their concentrations in micrograms/milliliter were phytohemagglutinin A (PHA) 5, 10, 50; concanavalin A (ConA) 3, 12, 25, 50; and pokeweed mitogen (PWM) 0.25, 2.5, and 5. These concentrations were determined in pilot studies.

PHA and ConA activate T lymphocyte subpopulations and PWM activates B and T cells in humans. The whole blood procedure of Bloemena, et al, (1998), which uses less blood than traditional assays, was adapted and verified in the laboratory. Whole blood diluted 10 fold with phosphate buffered saline (PBS) was incubated in 96 well plates with the specified concentrations of the 3 mitogens or with no mitogen (control). Cultures were plated in replicates of 6. For the last 4 hours of a 72 hour incubation, the cells were pulsed with ^{3}H Thymidine then harvested onto glass fiber filters. The radioactivity incorporated counts per minute (CPM) was detected with a ß-plate counter. The replicates were averaged and the standard deviations calculated. The average values were plotted versus concentration of mitogen to determine maximal concentrations. (see ATTACHMENT #2)

Whole Blood Phenotyping

This procedure was used to enumerate the proportions of lymphocytes in whole blood that bear specific surface antigens which indicate cell type, function and activation state. A Becton-Dickinson (BD) flow cytometry consultant spent several days advising staff on the most recent procedures for analyzing human blood (Mandy et al, 1997).

The panel of antibodies (Table 1) was chosen to determine the basic concentrations of CD4+ lymphocytes as well to describe their status, e.g. CD45RA+ identifies naïve cells. In addition the percentages of CD4+ T cells, B lymphocytes and NK (natural killer) cells were determined.

Table 1: Monoclonal Antibodies used to identify Lymphocyte subpopulations

Antibody combination	Cells recognized	Significance
CD45-ECD[a]	Leukocytes/monocytes	Used together with forward and side scatter to gate an exclusively lymphocyte population
FITC / PE Ig-G1-/IgG2a-	None	Isotype control
CD4/CD8	T_h (CD4), T_c (CD8)	Two T cell populations; should be mutually exclusive
CD3/CD4	Total T cells (CD3)/T_h (CD4)	Total T cells and percentage that are T_4
CD45RA/CD4	Naive T/T_H (CD4)	Determination of whether CD4 cells are from the thymus ($CD45RA^+$) or from peripheral stores ($CD45RA^-$)
HLADR/CD4	Activated cells/ CD4+	Indicates whether CD4 cells are activated, memory cells
CD4/CD69	CD4 T cells/nuclear activation	Indicates whether the CD4 cells are in the process of cell division
CD57CD8	Suppressor lymphs/ T_c (CD8)	Indicates the number of suppressor lymphs able to down regulate the CD4 lymphs
CD19/CD16	B cells/NK	Indicates the identity of non-T-lymphs
CD3/ CD16 + CD56	Total T/total NK	Indicates the $Cd3^-$ NK population
CD4+beads [b]	Th (CD4)	Absolute count of CD4+ cells
CD3+beads [b]	T_{total} (CD4)	Absolute count of CD3+ cells

[a] added to every sample to allow gating with each sample

[b] This sample is prepared with precise volumes; Trucount (TM) BD tubes were included for CD3 and CD4 preparations to determine absolute numbers of these cells in the blood.

The originally proposed procedure was modified by using a lymphocyte common antigen (LCA), CD45 conjugated with the Phycoerythrin-Texas Red (ECD) fluorochrome. ECD does not interfere with the Fluoroisothyocyonate (FITC) or Phycoerythrin (PE) conjugated fluorochromes of the other antibodies. This antibody was added to every sample. Because LCA labels only leukocytes it was used to set an internal gate in each sample. When combined with forward and side scatter it was used to gate only lymphocytes. Use of this internal gating worked very well although the antibody was very expensive and had to be added to every tube.

An isotype control was also used to assess non-specific binding which was very low. The combination of CD4/CD8 in the same sample was carried out to be certain the appropriate gates were set. This sample served as an internal control for compensation since the two subpopulations, CD4+ and CD8+ cells should be mutually exclusive in peripheral blood.

Originally, the panel was standardized using normal blood, however the blood from cancer patients, both before and after treatment, did not give the expected patterns. The procedure was modified and the CD4/CD8 tube was used to establish gates. A CD Chex Plus ™ (Streck Labs), a stabilized preparation of normal human peripheral blood leukocytes, was run as a control. In order to determine total T CD4+ cells, BD Trucount™ tubes were used along with anti-CD4 and anti-CD3 antibody.

The percentage of each cell population (T_H, T_C, B, NK) was determined by flow cytometry using the Epics XL program. This percentage was used in combination with the differential CBC lymphocyte counts to determine the absolute number of cells in each category for those samples in which Trucount™ was not included.

Cytokine Production Assay

Cytokine production by activated lymphocytes provides a good assessment of functionality of the cells. The particular panel of cytokines produced indicates the functional subgroup to which the cells belong; e.g. T_H1 (Interferon gamma IFNγ, Interleukin IL-2) vs T_H2 (IL-4, IL-6). Whole blood was diluted as per the mitogen assay and incubated with a T cell mitogen PHA at 10 μg/ml for 48 hours and the culture medium collected and frozen. Assays were done for Interferon gamma IFNγ and Interleukin IL-6 as indicators of T_H1 and T_H2 cells, respectively. These same molecules also were assayed in plasma from the same subjects.

Apoptosis Assay

Apoptosis of isolated while blood cells was carried out with APO 2.7, a detection antibody for a 38 kilodalton mitochondrial membrane protein which is exposed on cells undergoing programmed cell death. The APO 2.7 expressing cells were detected by flow cytometry. Very low levels of apoptosis were consistently seen and no differences were observed over time or between groups. Therefore the use of this assay was discontinued.

Physical Testing (see Appendix #3)

After breast cancer treatment (chemotherapy and radiation) was completed, subjects underwent baseline physical testing which included cardiovascular fitness, upper and lower body muscular strength, lower body power, body composition, and anthropometric skinfold analyses. These tests were repeated at 3 and 6 months into the exercise or control period. This part of the study was carried out by the staff of the Penn State General Clinical Research Center.

Body Composition Assessment/Anthropometric Measures
The body measurements recorded were weight and height using a physician's scale, and various arm, waist, and leg circumferences using a tape measure. Skinfolds were measured with a caliper at various sites on the front and back of arms, upper thigh, upper back, side of lower leg, and stomach. (see Appendix #4) This data was used to determine percentage body fat and Body Mass Index (BMI) and hip to waist ratio.

Cardiovascular Testing
A graded exercise test performed on a cycle ergometer was used to assess peak oxygen consumption. Heart rate was monitored continuously via a 12 lead EKG and ratings of perceived exertion was recorded every 3 minutes. This test progressed to harder and harder levels of work every 2 minutes at which time subjects rated feeling of fatigue from a numbered scale ranging from very easy to very hard. In addition, blood pressure was recorded every 3 minutes using an arm blood pressure cuff and expired gases were collected each minute by metabolic cart. The test was supervised by a physician and was terminated in accordance with the American College of Sports Medicine guidelines.

Muscle Function Testing
Triceps were tested by determining the maximal force exerted onto a force pad. The best of three to five attempts on each side was recorded as the maximal effort for each participant. Biceps strength was measured via a one-repetition maximum arm curl. The participant was instructed to lift the dumbbell from their side to their shoulder. The KinCom strength testing machine (called an Biodex isokinetic tester) was used to measure quadraceps and hamstring strength across three different isokinetic speeds (30, 90, and 180 degrees per second). The highest force between 40 and 75 units was recorded for each of the hamstring and quadraceps speeds out of three attempts with rests in between. Hand-grip strength was tested using a grip strength dynamometer. The highest score of three repetitions was used. Vertical jump height was measured three times with about 2 minutes rest between jumps. The highest jump was used for analysis.

Every effort was made to ensure safety through technique instruction and familiarization (teach how to perform the tests), experienced personnel, warm-up and cool down (i.e., stretching and low intensity activity specific exercise), and practice, supervision, screening, and monitoring while testing.

Exercise Training (see Appendix #5)

Following a series of fitness evaluations, all participants in the exercise group underwent training sessions three times per week over 3 months. Initial planning was for group training; however, pilot testing proved this approach was not feasible due to continuous enrollment and subjects' schedules. Therefore, training was changed to one-on-one. The program consisted of a warm-up, resistance training, and aerobic exercise. Following the warm-up period (5 minutes of light aerobic exercise and stretching), the women participated in resistance training using elastic rubber bands (Flexband, Jumpstretch, Inc., Boardman, Ohio - www.jumpstretch.com) to increase strength and power capacity. During each session, the women completed 4 upper body exercises and 4 lower body exercises using these bands. The bands came in graduated resistances allowing for a progression in increased resistance over time. In some cases, the women preferred to use hand held dumbbells when performing the upper body strength exercises. The women began the program by completing 1 set of each exercise (8-12 repetitions) and then progressed to 3 sets of each exercise by the end of week four. A 60 second rest period was permitted between each set of the exercise. Following completion of the resistance training portion of the exercise session, the subjects completed aerobic training that began at 10-20 minutes and then progressed to 20 minutes at 60-75% of the functional capacity as determined by the exercise test. The average session ranged in length from 40 to 90 minutes. Following the initial 3 months of training, the women either continued to exercise with their trainer or on their own with a home-based program for an additional 3 months. During the first three months, exercise logs were maintained by the trainers. While exercising at home, women were asked to keep an exercise log to be regularly collected by their trainer and met with their trainer once every two weeks

HUMAN SUBJECTS REVIEW

The study protocol was reviewed and approved by the Institutional Review Boards of the Hershey Medical Center, Hershey, Pennsylvania and the Centre Community Hospital, State College, Pennsylvania. The study was also approved by the Office for Regulatory Compliance Institutional Review Board Biomedical and Biosafety Committees of the Pennsylvania State University at University Park, Pennsylvania. Informed consent forms (see Appendix #6) were developed to meet the requirements of each of the three locations and approved by the Boards. One adverse event report was reported to the IRB at University Park related to EKG abnormalities observed during physical testing which caused the subject no distress. The protocols were not changed.

SUBJECT RECRUITMENT

Initially, recruitment efforts were within the Centre County region through the associate investigators, Dr. Aaron Bleznak, Dr. Richard Dixon, and Judy Underwood of Centre Community Hospital and Cancer Center. After receiving official approval from Hershey

Medical Center to conduct the study at that location for control subjects, recruitment efforts were undertaken in that area.

Until recruitment ended in January of 2002, extensive advertising and promotional efforts were made to reach both the general public and the medical community as follows:

General Public

Newspapers/TV/Radio/Web

Announcements –

Campus Public Information weekly newspaper, Intercom, November 2000.
Centre Daily Times health calendar section each Monday.
C-NET (public service television station)
University sponsored Health Web, "Health Matters"
Hershey local radio station.

Advertisements-

Centre Daily Times Essentials Section, on Mothers Day weekend (2001) and during Breast Cancer Awareness month, October 2001
Harrisburg Patriot in November, 2000 May, 2001, October 2001.
The Sun and The Hershey Chronicle, in May 2001, October 2001.
Altoona, Lewistown, Elizabethtown papers October 2001.
Several of the papers ran the ads in special sections devoted to health and/or breast cancer.
Relay for Life American Cancer Society (June, 2001) program

Flyers and Brochures (see Appendix #7 for sample flyer) - One page flyers and brochures with versions for controls and exercisers were produced as well as a small "bookmark" for easy referral.

These were placed in public sites (i.e. library, grocery stores, senior citizens centers), health care facilities(clinics, hospitals, physician waiting rooms, chemotherapy and radiation suites), exercise locations (health clubs and YMCA's), health and church fairs.
These were also distributed in person at events and to local women's health and support groups such as the display/information table at State College Breast Cancer Awareness Day, October 2001.

The local American Cancer Society and the "Reach for Recovery Group" a group of breast cancer survivors who visit women in their homes shortly after surgery to provide emotional and medical support both endorsed the study and distributed information.

Medical Community

The PI and other project staff met with and gave presentations to health care providers who were periodically given copies of abstracts submitted to organizations for annual meetings as well as updates on the current numbers in the study. Regular contact was maintained with the physicians and the chemotherapy nurses at the physician offices in the local area via phone calls, e-mails, and personal visits. In addition, to facilitate provision of information to possible study participants, "bookmarks" describing the study were distributed which could, for example, be kept in a physician's coat pocket.

A significant recruitment effort was initiated in the fall of 2001 with letters accompanied by packets of brochures and hand outs, sent to over 30 oncology services and physicians in the Centre county and Altoona, Lewistown, and Hershey/Harrisburg areas. Follow-up contacts were made.

Sister Thea Krause and Barbara Gutch, administrators of the newly formed Penn State Cancer Institute, a consortium including Centre Community Hospital, Hershey Medical Center and Lehigh Medical Center assisted in information distribution and recruitment.

Recruitment Difficulties

While these recruitment efforts provided some subjects, in the State College area, the principal source of subjects for the study was direct local physician referral. In Hershey, newspaper advertisements were the primary recruitment source.

Recruitment was one of the most unexpectedly difficult aspects of the study. Because of the incidence level of breast cancer in the area, this difficulty was not foreseen. But the women who volunteered were special in that many of them made a commitment to the study at a very difficult time in their lives. It was due to recruitment difficulties that this study was extended for one year with some supplemental funding.

Recruitment Procedures

In Centre County, Drs. Dixon and Bleznak met most of the women who underwent chemotherapy for breast cancer. If a potential subject was to receive chemotherapy, the physician or a staff member briefly explained the study and if she was interested, received her consent to be contacted. Her name, home number and certain basic information was given to the study coordinator who contacted her to discuss the study in more detail.

Eligibility was determined if the individual met the following criteria: 1) had documented breast cancer, 2) was scheduled to undergo or had undergone chemotherapy treatment within the past 5 months, 3) was able to perform normal daily activities, 4) received approval from her physician to participate in the program, and 5) less than 80 years old.

Of the women contacted by the study coordinator for information, many did not qualify for the study usually because they had been finished with chemotherapy for more than a year. Other reasons for disqualifying were taking medication which effected the immune system, or inability to commit to the length of study.

Eligible women were sent a packet of information, including an informed consent, which they were asked to read. A meeting time was arranged usually at the physician's office or at the GCRC at Penn State University before an appointment. At that time any

additional questions were answered and the informed consent was signed. The subject was then asked to provide a blood sample and complete three questionnaires. The blood was drawn at either the physician's office or at the GCRC and brought back to the department laboratory for analyses. CBC's and blood chemistry analyses were usually carried out through the GCRC.

When a control subject was recruited from the Hershey area, the collaborators at Hershey Medical Center met with the subject to review the informed consent. The blood was drawn at the Hershey GCRC where the CBC was completed and two heparin vials were sent overnight to University Park for the assays.

DATA ANALYSIS

Statistical databases were created in SPSS 10.0. A coding scheme was written to analyze the physical activity and quality of life questionnaires as suggested by the developers. Paired t-tests were used to examine differences in immune function, quality of life, physical activity, and physical function in the exercise and control groups over time. In addition, the nutritional data has been analyzed using Nutritionist Pro and the results are being entered into a database for further analyses. Upon completion of the program, exercise subjects were provided a Participant Summary Report with general information and their specific data along with some analyses.(see Appendix #8)

Missing Data

Blood

Some blood sample assays are incomplete or missing entirely. Several scheduled blood draws at the University Park GCRC did not occur due to subjects presenting with ports which the GCRC staff is not equipped to handle. Some attempts to draw blood were unsuccessful or the amount drawn was inadequate for analysis purposes. A few blood samples sent overnight from Hershey did not arrive or did not arrive till the sample was unusable. In these cases, CBC data was completed at Hershey. In several instances, when blood was drawn at medical offices or labs, the CBC was not completed or the information was not provided. CBC reports from different sources varied in content. Occasionally assays problems occur due to equipment failure, cell contamination or technical errors.

Physical Testing

Some subjects arrived for physical testing with physical problems such as respiratory complications, or knee injuries which precluded part or all of the testing. When possible testing was rescheduled. Some subjects terminated testing or testing was terminated prematurely due to abnormalities noted via the monitoring.

Self-reports

Some questionnaires were not returned or returned incomplete, or blank

Results

Subject Recruitment/Enrolment

The study protocol includes five possible data collection points -
Time 1 (T1) - Pre-chemotherapy
Time 2 (T2) - Pre-radiation therapy
Time 3 (T3) - Post-Therapy Baseline Pre-exercise
NOTE: For women not receiving radiation, T2 = T3
Time 4 (T4) - Mid-Study/ Mid-Exercise
Time 5 (T5) - End-Study/ End-Exercise

Recruitment was ongoing until January of 2002. Our goal was to have 35 exercisers and 35 controls complete the study protocol. The final subject count was 28 exercisers and 21 controls – women who completed the baseline/pre-exercise data collection and at least the mid-exercise /mid study point. Of the 28 exercisers, 21 completed pre-, mid- and end exercise data points and 7 completed pre- and mid-exercise data points. Of the 21 controls, 16 completed the baseline, mid and end study and 5 completed baseline and mid. A number of women (approximately 10) after initial recruitment, decided not to participate and dropped out of the study prior to completing any data points.

Although the ideal point for entry into the study was prior to chemotherapy, a number of the women started participating after completion of chemotherapy (T2) or after completion of radiation (T3). A number of women who wanted to be exercisers but could not regularly come to the exercise facility to work with the trainer were asked to be controls. Only a few agreed.

For 15 other women referred to as "patients," one or more data points up to and including baseline (T3) were completed. Because no information was collected on these patients after the intervention point, this data set will be used for additional analysis of the effects of treatment on blood components. It was not included in this part of the study. From all participants, 25 comparative samples are available pre- and post chemotherapy treatment, 23 pre- and post-radiation treatment and 4 pre-chemo and post-radiation.

Three blood samples were drawn from each of 13 healthy subjects matched for age and BMI over approximately a six month period. The data from this group serve as a reference for the amount of immune parameter change over time in normal healthy individuals.

Subject Description

The 28 exercisers and 21 controls, had a mean age of 50.12 years and an age range of 29 to 71 years. There was no significant difference between the drop-outs, patients, controls, and exercises for marital status, age; or between exercisers and controls for baseline (T3) BMI, or baseline body fat percentage. (Table 2 and 3) However, there was a difference between exercisers and controls in educational level and type of job. This difference

could be due to two factors: the job flexibility required for exercisers to complete sessions on campus with the trainer may be related to higher level jobs and thus to higher education levels; and an enticement for control subjects recruited at Hershey was payment which might be more attractive to women in lower paying jobs. The ethnicity of all of the subjects is Caucasian with the exception of one Hispanic woman.

Table 2: Subject Physical Characteristics

Variable	Group	N	Mean /SD	Sig
Age (years)	Control	21	52.3 ± 9.2	NS
	Exercise	28	48.5 ± 10.6	
Weight (kg) Baseline T3	Control	21	72.4 ± 11.2	NS
	Exercise	28	73.6 ± 13.7	
Body Mass Index	Control	21	26.63 ± 4.13	NS
	Exercise	28	26.67 ± 5.35	
Percent Fat Baseline T3	Control	20	33.03 + 8.00	NS
	Exerciser	28	33.45 + 8.07	

Table 3: Subject Demographics

Variable	Total Sample		Exercisers		Controls	
	N	%	N	%	N	%
Marital Status						
Married	37	75.6	22	78.6	15	71.4
Widowed	3	6.1	1	3.6	2	9.5
Single	6	12.2	3	10.7	3	14.3
Divorced/Separated	3	6.1	2	7.1	1	4.8
Total	49	100	28	100	21	100
Job Type						
Business	12	24.5	8	28.6	4	19.0
Non-Business Professional	17	34.7	11	39.3	6	28.6
Retail/Sales	3	6.1	0	0.0	3	14.4
Other	6	12.2	2	7.1	4	19.0
Unemployed/Retired	11	22.5	7	25.0	4	19.0
Total	49	100	28	100	21	100
Employment Status						
Full-Time	29	59.2	18	64.3	11	52.4
Part-Time	6	12.3	3	10.7	3	14.3
Unemployed/Retired/Other	14	28.5	7	25.0	7	33.3
Total	49	100	28	100	21	100
Education						
High School	9	18.4	3	10.7	6	28.6
Some College	11	22.4	4	14.3	7	33.3
College Degree	12	24.5	11	39.3	1	4.8
Graduate School	9	18.4	6	21.4	3	14.3
Other/Not Given	8	16.3	4	14.3	4	19.0
Total	49	100	28	100	21	100

Of the 49 exercisers and controls, most were diagnosed with stages I-III breast cancer (Table 4). Surgical treatment varied with 35 percent having mastectomies and 55 percent having lumpectomies. The majority of the women underwent similar chemotherapy treatment that consisted of. 4 cycles of Adriamycin/ Cytoxan (51%) while 11 women (22.5%) received treatments of A/C plus taxol. This regimen takes approximately 3 to 4 months to complete. However, others (14.3%) were treated with a cocktail of CMF (Cytoxan, Methotrexanate, 5-Flourauricil) for about 6 months.

In addition to chemotherapy treatment, a majority of the women (71%) underwent radiation, usually for 4 to 7 weeks, as part of their breast cancer and some of the women underwent additional surgical treatment (e.g. reconstruction) that prolonged their treatment or ended their participation in the study prematurely.

Table 4: Breast Cancer characteristics for Exercisers and Controls

Variable	Total Sample		Exercisers		Controls	
	N	%	N	%	N	%
Stages						
I	11	22.4	6	21.4	5	23.8
II	29	59.2	19	67.9	10	47.6
III	4	8.2	2	7.2	2	9.5
Unable to Stage	5	10.2	1	3.5	4	19.1
Total	49	100	28	100	21	100
Type of Surgery						
Lumpectomy	26	55.0	15	53.6	11	52.4
Mastectomy	21	35.0	11	39.2	10	47.6
Unknown	2	10.0	2	7.2	0	0.0
Total	49	100	28	100	21	100
Chemotherapy Treatment						
A/C	25	51.0	13	46.4	12	57.2
A/C plus Taxol	11	22.5	6	21.4	5	23.8
CMF	7	14.3	5	17.9	2	9.5
Other	6	12.2	4	14.3	2	9.5
Total	49	100	28	100	21	100
Radiation Treatment						
Yes	35	71.4	19	67.9	16	76.2
No	14	28.6	9	32.1	5	23.8
Total	49	100	28	100	21	100

Because of the different treatment regimes, the length of time women were in the study varied from a low of 3.5 months for T3 to T5 to a high of 19 months for T1 to T5 with an average of about 10 months. The intervals between baseline/pre-exercise, mid-exercise/mid-study and end-study/end exercise were to be 3 months each. However, the average was 4 months primarily due to individual schedule difficulties related to factors such as vacations and appointment availability.

Cardiovascular Function

The cardiovascular function measures related to respiration (Ve), oxygen intake (VO2) and heart rate for the exercise group increased significantly, or nearly so, over the course of the exercise training (Table 5). For example, the absolute VO2 max at baseline was 1466±252 and significantly increased to 1624±312 after six months of training. The control group had an average baseline VO2 max of 1365±346, not significantly different from the exercisers' mean baseline value. However, the values for the control group did not increase after 6 months.

Table 5– Cardiovascular Measurements (*=p≤0.05)

	Exercisers			Controls		
Variable	Mean ± S.D.	N	Sig. of Diff. from Baseline	Mean ± S.D.	N	Sig. of Diff from Baseline
[1]Ve (litre/min) Baseline	63.1±13.4	28		59.5±14.1	19	
Ve (l/min) Mid-study	69.8±14.2	27	0.012*	58.9±14.1	17	0.860
Ve (l/min) End-study	71.9±15.5	19	0.018*	65.2±11.5	12	0.465
[2]VO2(ml/min) Baseline	1466±252	28		1365±346	19	
VO2(ml/min) Mid-study	1544±305	27	0.080	1356±345	18	0.559
VO2(ml/min) End-study	1624±312	19	0.019*	1428±295	13	0.380
[3]VO2/KG (ml/kg) Baseline	20.4±4.5	28		19.5±5.6	19	
VO2/KG(ml/kg) Mid-study	21.3±4.1	27	0.126	19.1±5.1	18	0.740
VO2/KG(ml/kg) End-study	22.9±4.5	19	0.052	19.9±4.7	13	0.922
Peak Heart Rate - Baseline	163.6±18.2	28		162.7±16.0	19	
Peak Heart Rate Mid-study	167.7±12.7	27	0.344	159.7±19.2	17	0.075
Peak Heart Rate End-study	172.5±10.8	19	0.072	162.5±17.6	13	0.287

NOTE: Comparison for baseline to end were appropriately adjusted for sample size.

[1] = Ve is a measure of ventilation or respiratory rate based on breaths taken per second expressed as litres per minute.

[2] = Absolute VO2 max is the maximal oxygen uptake expressed in milliliters per minute.

[3]= Relative Vo2 max is absolute VO2 divided by body weight expressed as milliliters per kilogram.

Strength Measurements

The four measurements of upper body strength - left and right hand grip and bicep and tricep curl – improved significantly over time for the exercise group (Table 6). The baseline values for the control group were not different from the baseline for the exercise group and did not change significantly over time. In fact, on several measures there was a decrease for controls.

Table 6 – Strength Measurements (*=p≤0.05)

	Exercisers			Controls		
Variable	Mean ±S.D.	N	Sig. of Diff. from Baseline	Mean ± S.D.	N	Sig. of Diff from Baseline
Left Hand grip (kg) Baseline	23.39±7.50	27		23.00±4.36	19	
Left Hand grip (kg) Mid-study	25.70±6.24	27	0.011*	23.24±5.31	19	0.747
Left Hand grip (kg) End-study	27.00±6.32	20	0.004*	21.92±4.87	13	0.688
Right Hand (kg) Baseline	25.65±6.80	26		25.11±4.22	19	
Right Hand (kg) Mid-study	27.85±6.37	26	0.008*	25.37±4.73	19	0.696
Right Hand (kg) End-study	29.08±6.76	19	0.002*	23.69±3.98	13	0.297
Bicep Curl (lb) Baseline	17.02±4.33	28		16.58±3.54	18	
Bicep Curl (lb)Mid-study	18.69±4.29	28	0.003*	15.83±4.08	18	0.121
Bicep Curl (lb)End-study	19.12±3.48	19	0.002*	16.85±3.47	12	0.518
Tricep (lb) - Baseline	23.01±5.70	28		22.02±5.80	19	
Tricep (lb) -Mid-study	24.62±6.18	28	0.028*	20.79±4.95	19	0.337
Tricep (lb) -End-study	25.59±7.60	20	0.002*	20.90±6.80	13	0.620

NOTE: Comparison for baseline to end were appropriately adjusted for sample size.

Quality of Life

The mean overall score for the health-related quality of life (FACT) questionnaire was significantly higher for the exerciser group compared to the control group (Table 7) at the end of 6 months. The FACT social well-being subscale also had a significantly higher mean score for the exercise group compared to the control group at the end of the study.

Table 7 – FACT Questionnaire

Variable	Group	Number	Mean /SD	F	Sig
FACT score	Control Exercise	17 26	93.63 ± 3.23 100.65 ± 1.69	4.0	0.05
Social well-being score	Control Exercise	15 19	21.67 + 4.06 24.88 + 2.81	7.15	0.01
Emotional well-being score	Control Exercise	15 19	16.55+ 3.44 17.79 + 2.78	1.35	NS
Physical well-being score	Control Exerciser	15 19	26.02 + 2.10 26.63 + 1.11	1.15	NS
Functional well-being score	Control Exerciser	15 18	22.79 + 4.32 24.46 + 6.50	0.73	NS
Fatigue score	Control Exerciser	14 19	45.16 + 7.05 45.92 + 11.49	0.05	NS

Cytokine Levels

Plasma cytokines and cytokines produced by stimulated lymphocytes were determined by ELISA. IFNγ and IL-6 were assayed as indicators of T_H1 and T_H2 activity. In addition, plasma levels of both of these had been measured in cancer patients in other studies. At the time of this report, samples from 25 exercisers and 12 non-exercisers have been measured. The remaining samples are frozen. The last samples were collected in August and will be assayed as a batch within the comimg weeks. Samples from 13 healthy (non-cancer) women, age and BMI matched to the study participants, were also assayed. The plasma and lymphocyte secreted IFNγ and IL-6 levels for these women did not vary significantly over 6 months. In comparison, the breast cancer patients had elevated levels of lymphocyte secreted IFNγ and IL-6. IFNγ production, but not IL-6, significantly decreased by 6 months. In contrast, plasma levels of both cytokines were lower than healthy women and did not change significantly over time. Comparisons between exercisers and non-exercisers will be made once all the samples are analyzed. In addition, IL-7 has been implicated in the restoration of the immune system following chemotherapy. This cytokine will be assayed in the frozen samples of all the participants

Lymphocyte Subpopulations

The mean absolute number of total T lymphocytes increased in both control and exercise groups (Table 8). Likewise the CD4 and CD8 values increased with time. However, at the end of 6 months, the average of the absolute numbers of CD4 cells still did not reach the normal range of 800 to 1200. B cells recovered more rapidly that T cells and nearly reached the normal range (200 – 400). NK cells also increased in both groups. The exercise and control groups were not significantly different for these parameters. **However, the percentage of naïve (CD45+RA+) CD4 T cells increased significantly in the exercise group but not in the control group.**

Table 8 – Lymphocyte Subpopulations – Mean number of cells/ul blood *p<0.05 compared to baseline

Data Point	CD3+ Total T		CD4+ T Helper		CD8 T cytotoxic		CD19 B cells		NK Natural Killer		CD4+CD45RA+ Naïve CD4+	
Group	E	C	E	C	E	C	E	C	E	C	E	C
Pre-exercise/ Baseline	717	731	488	497	257	270	49	98	78	64	108	105
Mid-study	811*	830*	564*	570*	311*	343	81*	173*	97	120*	131	103
End study	828*	866*	549*	577*	377*	406	115*	143*	102	108*	163*	113

E = exercisers C = control

Key Research Accomplishments

Thefinding that an exercise intervention program can increase naïve (new) CD4 lymphocytes in the circulation.
Exercise led to increased quality of life and physical fitness in a population recovering from chemotherapy.
Selection of questionnaires and developing analysis procedure
Selection, modification and pilot testing of blood assays
Determining components of the physical testing
Developing the exercise training program
Creating and refining forms for all aspects of the study record keeping
Soliciting and receiving the cooperation and participation of the medical community in State College and Hershey, Pennsylvania
Developing appropriate procedures for the cooperative efforts of both the Hershey and Penn State General Clinic Research Centers
Receiving and maintaining approval of the three IRB's for the Study
Creating and having the consent forms approved
Recruiting and training staff
Designing an appropriate database and spreadsheets.
Development of recruitment materials
Implementation of recruitment efforts
Assembling and giving presentations
Using study as basis for degrees, experience and training
Cleaning data and initial data analysis

Reportable Outcomes (listed chronologically)

June 2000 Era of Hope, Department of Defense Breast Cancer Research Program Meeting.

Mastro, A. "The Use of Exercise to Increase CD4(+)T Lymphocytes following Chemotherapy Treatment for Breast Cancer"

March 2001 AACR Annual Meeting Poster Presentation

Mastro, A., Williams, N., Kraemer, W., Orsega-Smith, E., Perry, M., Dixon, R., Bleznak, A., & Underwood, J. (2001). Exercise, quality of life, and the recovery of CD4 Lymphocytes following chemotherapy for breast cancer. American Association for Cancer Research Scientific Proceedings, 42, 331.

June 2001 American College of Sports Medicine Annual Meeting Poster Presentation

Orsega-Smith, E., Williams, N., Perry, M. Mastro, A., Kraemer, W., Bleznak, A., Dixon, R., & Underwood, J. (2001). Fatigue, quality of life, and physical function after chemotherapy for breast cancer. Medicine & Science in Sports & Exercise, 33(5), S306.

June 2001 American College of Sports Medicine Annual Meeting Paper Presentation

Perry, M., Mastro, A., Orsega-Smith, E., Miles, M., Kraemer, W., & Willimas, N. (2001). Exercise training and immune function following chemotherapy for breast cancer. Medicine & Science in Sports & Exercise, 33(5), S77.

June 2001 Hershey Medical Center, General Clinical Research Center Presentation

Andrea Mastro, Ph.D., presentation "Breast Cancer and Exercise"

2002 American Association for Cancer Research 93rd Annual Meeting

Mastro, A.M., Williams, N.J., Ford, J., Fuener, K., Orsega-Smith, E., Kraemer, W.J.; Bleznak, A.D., Dixon, R.H., Underwood, J., Miles, M., Wagner, K. (2002). IL-6 and Interferon-Gamma Levels Following Chemotherapy for Breast Cancer.

April 2002 Penn State Undergraduate Research Exhibition

April 2002 National Conference for Undergraduate Research, Whitewater Wisconsin,

Jen Ford - undergraduate research assistant and Penn State Graduate May 2002 "Can Exercise Effect Cytokine Levels in the Blood of Breast Cancer Patients Undergoing Chemotherapy?"

April 2002 Pennsylvania State University, University Park Campus, Noll Laboratory Seminar Series,

Andrea Mastro, Ph.D., "Metastatic Breast Cancer and Osteoblast Apoptosis"

August 2002 at the 10th Annual Penn State McNair Summer Research Conference. November 2nd, 2002 at the Delevan, Wisconsin McNair Conference.

Melanie Bombar - McNair Post-Baccalaureate Achievement Program summer research program, "The Effects of a Supervised Exercise Program on Activities of Daily Living In Women Recovering From Breast Cancer After Chemotherapy"

Post-doctoral experience for Beth Orsega-Smith, Ph.D.

Practicuum work for Beth Baker MS degree from Indiana University of Pennsylvania

Masters thesis data for Mike Perry
Training of Keira Fuerner as a lab technician
(see ATTACHMENT #9 listing paid personnel including students)

CONCLUSIONS

Summary and Conclusions

This study has shown that a 6 month exercise program benefited women recovering from chemotherapy in several ways that were not seen in a comparable group of women who were not enrolled in the formal exercise program:

- The percentage of naïve (CD45 RA+) T helper (CD4+) lymphocytes increased significantly.
- There was a significant increase in cardiovascular function.
- There was a significant increase in upper body strength.
- There was a significant increase in self-reported quality of life.

Discussion

The primary hypothesis was that an exercise intervention program would increase the recovery of CDA, T helper lymphocytes in women following chemotherapy for breast cancer. We found that the CD4 lymphocytes increased but in neither the exercise or control groups did the CD4 levels reach the normal population range at the end of the 6 months. A Previous publication had shown that even at 18 months, normal levels had not been reached. However, the exercise group showed a significant increase in the percentage of naïve CD4 lymphocytes. These naïve cells are produced in the thymus while the remaining CD4 cells come from the peripheral circulation. These results suggest that exercise can stimulate thymic activity. Exercise is known to cause production of several cytokines and hormones. It is likely that one or a combination of such molecules can act on the thymus. It will be of interest to pursue this novel finding. Identification of this factor and mechanism could lead to ways to enhance this activity in cancer patients and in other immunosuppressed individuals.

In addition, the results of this study demonstrated that an exercise program significantly improved the physical strength, endurance and well being of the women in the exercise group compared to the control group, who were not in the formal exercise program. While these results were not unexpected, they so confirm the many benefits of exercise for a population suffering from the social and psychological as well as physical side effects of chemotherapy.

The feedback from the participants in the study was very positive. Several social gatherings were held during the course of the study to allow participants to meet each other and to learn of the progress of the study. The women were given a set of Jumpstretch bands and many have continued their exercise program.

The analyses to date have been minimal because data collection only ended in early August 2002. Efforts are still ongoing to try to obtain several missing data items. Also the complexities of analysis to appropriately address missing data need to be examined. Our statistical consulting center will be helping with analyses. We will examine the blood chemistry changes related to chemotherapy, radiation therapy and the combination. Mitogen data will be examined in more detail. The plasma and secreted cytokines, IL-6, IFNγ and IL-7 will be measured in remaining samples. The data from the healthy controls will be used to determine the normal pattern of the phenotype panel over time.

A shortcoming of the study was the realtively low number of subjects and their homogeneity. Nevertheless, the N values were very reasonable for an intervention study and the results do provide more exact definition for the research question so that data collection can be refined and focused to those areas of specific interest for application to a larger subject pool. Another shortcoming was the lack of random assignment. Women were largely self-selected . It is difficult to have a placebo for exercise. However, because we did not carry out group training, we did not have to be as concerned about a group support effect.

In summary, this study addressed an important health-related immune problem that had not been examined previously by others in breast cancer research. It tested an intervention for women with breast cancer that was carried out following chemotherapy treatment. A search of the literature from 1990-2002 revealed no other comparable studies examining exercise interventions as a way to increase immune cell recovery following chemotherapy. This study examined the mechanism of the recovery of the immune cells after depletion by chemotherapy including the source of the cells (naïve or memory), state of activation, cycling or apoptotic, and the role of cytokines. It also examined the quality of life over the time from pre-chemotherapy through completion of exercise intervention following chemotherapy for breast cancer. This exercise intervention was much longer than most studies that have examined physical activity and quality of life in cancer patients.

The results indicated expected increases in physical fitness and quality of life. The novel finding was an increase in naïve (newly matured) CD4+ (T helper cells) in women who took part in the exercise program. The results from this study may help to develop a non-invasive, low cost therapeutic intervention for women recovering from breast cancer. This intervention can be carried out in a variety of settings therefore allowing replication in both the community and clinical settings. It is hoped that this intervention may help to provide scientific clues to the mechanism by which exercise affects the immune system as well as enhance the quality of life.

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APPENDICES

Appendix #1- blood assay protocol
Appendix # 2- mitogen data and chart
Appendix # 3- physical testing protocols
Appendix # 4- skin fold measures
Appendix # 5- exercise protocols
Appendix # 6 - sample informed consent
Appendix # 7 - sample flyer
Appendix # 8 - participant summary
Appendix # 9 – personnel

WHOLE BLOOD MITOGEN ASSAY
DATE: **DONOR:**

PRINCIPLE:
DILUTED WHOLE BLOOD IS CULTURED WITH ONE OF MORE OF THE LECTINS (MITOGENS) FOR 72 HOURS, AT WHICH TIME THE MAXIMAL EFFECT OF DNA SYNTHESIS CAN BE SEEN. THE MEASUREMENT OF THIS DNA SYNTHESIS IS ACCOMPLISHED BY PULSING (LABELING) THE CULTURES WITH TRITATED THYMIDINE, A NUCLEOTIDE PRECURSOR THAT IS INCORPORATED INTO DNA. THE COUNTS MEASURED REPRESENT A MEASURE OF THE LYMPHOCYTE RESPONSIVENESS TO NONSPECIFIC ANTIGENS.

PROCEDURE:

1. PREPARE MITOGEN DILUTIONS AND PLATE (96 ROUND BOTTOM)ACCORDING TO CHART BELOW:

MITOGEN PREPARATION:
PHA:
A = 200ul STOCK (1mg/ml) + 1800ul of RPMI
CON A
B = 200 ul STOCK (1mg/ml) + 1800ul of RPMI
PWM
C = 20ul STOCK (1mg/ml) + 1980ul of RPMI

	SAMPLE	CELLS	BUFFER RPMI - uL	MITOGEN uL
A1-6	BLANK		200	
A7-12	BLANK		200	
B1-6	CONTROL	100uL	100	0
B7-12	PHA 50		0	100 A
C1-6	PHA 10		80	20 A
C7-12	PHA 5		90	I0 A
D1-6	CON A 50		0	100 B
D7-12	CON A 25		50	50 B
E1-6	CON A 12		75	25 B
E7-12	CON A 3		94	6 B
F1-6	PWM 5		0	100 C
F7-12	PWM 2.5		50	50 C
G1-6	PWM .25		95	5 C

2. DILUTE HEPARANIZED BLOOD 1.3ML BLOOD TO 11.7ML RPMI COMPLETE MEDIA.
3. PIPET 100UL OF DILUTED BLOOD INTO EACH WELL OF MITOGEN DILUTIONS. AS A CONTROL, PIPET 100UL INTO 6 WELLS CONTAINING RPMI COMPLETE MEDIA ALONE.
4. COVER 96 WELL PLATE WITH LID AND INCUBATE IN A 37°C 5%CO_2 INCUBATOR FOR 72 HOURS.
5. AT 72 HOURS, REMOVE PLATES AND ADD 10UL OF TRITATED THYMIDINE ([ ] = 100uCi/ML (1:10 DILUTION OF STOCK)) TO EACH WELL. RETURN TO INCUBATOR FOR 4 HOURS.
6. AT EXACTLY 4 HOURS, HARVEST THE CULTURES USING A MULTIPLE SAMPLE HARVESTOR.
7. ALLOW TIME FOR FILTERMAT TO AIR DRY. MAT MUST BE COMPLETELY DRY!
8. PLACE MAT INTO BAG AND ADD 10 ML SCINTILLATION FLUID. SEAL BAG.
9. COUNT FILTERMAT IN BETAPLATE COUNTER COUNTING EACH WELL FOR 2 MINUTES.

MEDIUM:
450ML RPMI
50ML FETAL BOVINE SERUM
2.5ML PEN/STREP
1 ML M L-GLUTAMINE

FOR CYTOKINE ASSAY: 24 WELL PLATE
A1 – 2ML DILUTED BLOOD
A2 – 2ML DILUTED BLOOD +
10ul PHA (1mg/ml)
AT 48HRS, COLLECT SAMPLES INTO EPPENDORF TUBES AND SPIN. COLLECT SUPERNANT INTO 1ML EPPENDORFS AND PLACE IN –80° FREEZER

LYMPHOCYTE PHENOTYPING VIA FLOW CYTOMETRY

Objectives:

a. to stain cells with fluorescent antibodies that bind distinguishing proteins on the cell's surface.
b. To determine the absolute number of certain blood cells.

Accuracy is critical!!

Reagents

1. PBSA is PBS without the Ca and Mg.
2. 1% formadehyde is prepared by taking 5 ml of the 20% stock (Tousimis in Frig) and adding 95 ml of PBSA. Store in brown bottle in Frig.
3. Optilyse solution use as is (Coulter product)
4. FACS lyse from BD is 10X. Use at 1X. Dilute 1:10 with water.
5. ACK lysing solution is made in the lab. (Mishel and Shiggi book).
6. Dilute some bleach 1:10 to put in a beaker in the hood to collect tips.

General Procedures

1. Pipette original blood by back piptting to get accuate volumes. Try to use the same pipettors and make sure they are certified.
2. You can vortex the samples after adding Ab but do so briefly and at medium speeds.
3. Clean out the vauum flask from blood at the end of the day. Do not leave bleach in the flask or it will ruin the pump. Also rejuvenate the drying reagent in the vacuum trap and change the filter periodically.

LYMPHOCYTE PHENOTYPING VIA FLOW CYTOMETRY

1. Collect blood in Na heparin treated Vacutainer tube (green top). Place on rocker at room temperature until analysis.

2. Prepare individual flow tubes in a dark area (eg laminar flow hood with light off):
   Label 12 x 75 mm polystyrene tubes 1-9, 12, 13 and CD Chex
   Label 2 BD TruCount tubes 10 and 11

   Add 5µl CD45-ECD to each tube.
   Add 5µl of each antibody to the tubes as follows:

	ECD	FITC(green)	PE (Orange)
1.	ECD	CD4	CD8
2.	ECD	IgG_1	IgG_{2a}*
3.	ECD	CD3	CD4
4.	ECD	CD45RA	CD4
5.	ECD	HLA-DR	CD4
6.	ECD	CD4	CD69
7.	ECD	CD57	CD8
8.	ECD	CD19	CD16
9.	ECD	CD3	CD16+56*
10.	ECD	CD3	
11.	ECD	CD4	
12.	ECD	CD3	
13.	ECD	CD4	
CD Chex	ECD	Varies**	

   * These antibodies are Simultest antibodies. The bottle contains both the FITC-conjugated and the PE-conjugated antibodies. Therefore, pipet only one 5µl aliquot per tube.

   ** The antibodies used to stain CD Chex Plus should be alternated. Just be sure to use one FITC-conjugated and one PE-conjugated antibody per tube (staining for different markers).
   Example: CD4-FITC and CD8-PE
   CD19-FITC and CD4-PE

3. Set the tubes aside in a dark area.

4. Place 1 ml blood + 1 ml PBS A in a 12 x 75 mm tube. Vortex or invert to mix. Back pipette the blood. Volumes are critical.

5. Place ~600µl whole blood in a second 12 x 75 mm tube.

6. The tubes will be processed in three separate batches as follows:

Tubes 1-9:

7. Add 100µl diluted blood to tubes(blood all the way to bottom of tube)
8. Incubate for 20 minutes at 4°C (refrigerator)
9. Remove from refrigerator and allow to come to room temperature (~1 minute)
10. Add 3 ml ACK Lysis Buffer to each tube
11. Spin at 1000 rpm for 4 min
12. Aspirate supernatant
13. Rinse with 1 ml PBS A
14. Spin at 1000 rpm for 4 min
15. Aspirate supernatant
16. Add 500µl 1% formaldehyde and vortex
17. Store at 4°C until flow cytometric analysis

Tubes 10 and 11:

7. Add 50µl whole blood to tubes
8. Incubate for 15 minutes at room temperature in the dark
9. Add 450µl 1X FACS Lysis buffer
10. Incubate at room temperature in the dark for 10 minutes
11. Store at 4°C until flow cytometric analysis

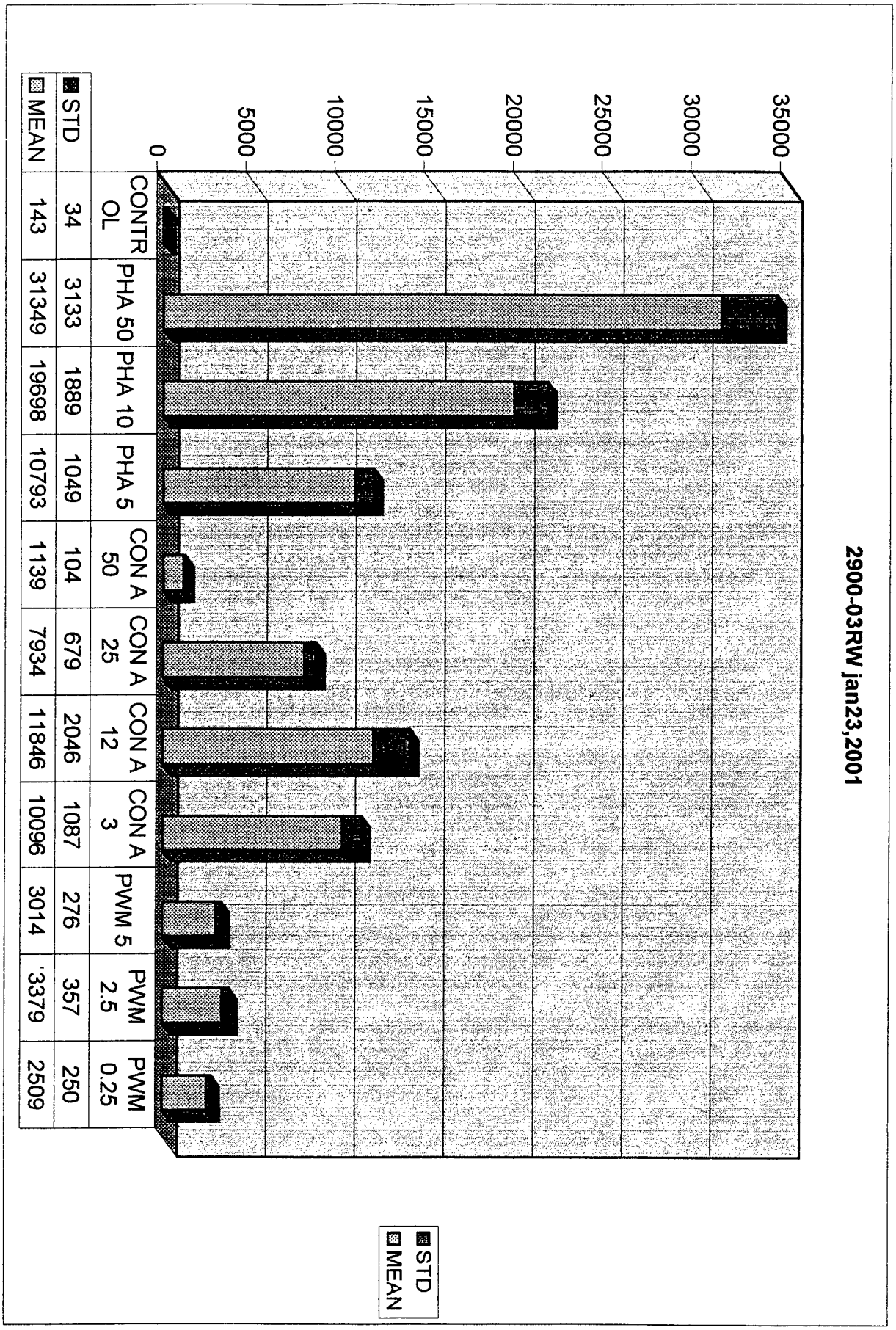
Tubes 12 and 13:

7. Add 100µl whole blood to tubes
8. Incubate for 15 minutes at room temperature in the dark
9. Add 500µl Optilyse C
10. Incubate at room temperature in the dark for 10 minutes
11. Store at 4°C until flow cytometric analysis
12. Just prior to flow cytometric analysis (no more than 2 hrs prior), add 100µl Flow Count Fluorospheres to tube (vortex spheres vigorously before taking aliquot)

CD Chex:

7. Add 100µl CD Chex Plus to tube
8. Incubate for 15 minutes at room temperature in the dark
9. Add 500µl Optilyse C
10. Incubate at room temperature in the dark for 10 minutes
11. Store at 4°C until flow cytometric analysis

Appendix # 2 Mastro, Andrea DAMD 17-98-1-8142
Mitogen Data and Chart



	CONTROL	PHA 50	PHA 10	PHA 5	CON A 50	CON A 25	CON A 12	CON A 3	PWM 5	PWM 2.5	PWM 0.25
STD	34	3133	1889	1049	104	679	2046	1087	276	357	250
MEAN	143	31349	19698	10793	1139	7934	11846	10096	3014	3379	2509

PENNSTATE

General Clinical Research Center

The Pennsylvania State University
211 Noll Laboratory
University Park, PA 16802-6901

(814) 865-7103
Fax: (814) 865-0351

Standard Operating Procedure - Bicycle Ergometer VO2 max test

Purpose: To determine a subject's cardiorespiratory fitness for inclusion into studies or for descriptive data.

Equipment: Monark bicycle ergometer, metabolic cart system, breathing apparatus, Polar heart rate monitor (for younger subjects), Marquette EKG machine (for older subjects)

Person Responsible: Exercise Physiologist, Study investigator (or grad student from study), Doctor (for older subjects), Nurse (if needed)

Procedure:

Explain procedure to subject
Place the Polar Heart Rate monitor on younger subject or attach ECG leads on older subject
Adjust seat height so that the subject's leg is slightly bent when pedal is at the bottom of the swing
Adjust handlebars for comfort of the subject
Place breathing apparatus on subject, including nose clip
Have subject warm-up for approx. four minutes
Set metronome for pace of 60 rpm, instruct subject to keep pace with it
Proceed with test using modified Astrand protocol
- *maintaining pace of 60 rpm throughout test
- *starting workload of 180 kgm (0.5 kp)
- *increasing workload by 180 kgm (0.5 kp) every two minutes until exhaustion

(The Astrand protocol is 150kgm(0.5 kp) with pedal speed of 50 rpm)
Record heart rate every minute for all subjects
Record blood pressure every two minutes for older subjects (> 40 for men, > 50 for women)
Record RPE every two minutes for all subjects
After max has been reached, loosen tension on the pedals and have subject cool down until their heart rate is approx. 120 for younger subjects or 100 for older subjects
Remove equipment from subject, who is free to leave if they are feeling well
Clean equipment

Breast Cancer Exercise Study
EXERCISE STUDY MUSCLE TESTING PROTOCOLS

ORDER OF TESTING:

Visit #1 =
- Medical Screening
- Bike protocol
- Body Composition

Visit #2 =
- Kincom (familiarization)
- Tricep (familiarization)
- Grip Strength (actual)
- Vertical Jump (actual)
- Bicep (familiarization)

Visit # 3 =
- Kincom (actual)
- Tricep (actual)
- Bicep (actual)

INSTRUCTIONS:

KINCOM PROTOCOL:

TRICEP MAXIMUM FORCE PROTOCOL

Computer Set-Up and Instructions:

- Connect Force Pad into the back of the Digital Myograph. If this is not connected, when you turn on the power the fault button will light up.
- The only buttons that should be pushed in besides the power button is **"SINGLE"** and **"PK FORCE"**. % DIFF and PRINTER button should NOT be pushed.
- The **START TEST** and **TEST 1** light must be lit before you can begin the test.
- When the administer says go, the subject will push on the force pad. The force will be shown on the screen. When the test is done, a beep will sound (End Test light will be lit). The subject can release their palm from the force pad and relax. The peak force for the specific time will be shown on the display mode.
- After the peak force has been recorded, press the **RESET** button. You may begin testing again when the **START TEST** and **TEST 1** light is lit.
- To change the time interval, move the number to the specific number you are testing.

Instructions to Subject:

Subject will be instructed to sit in the chair facing the administrator with their back straight (if there is a back to the chair, have subject sit with back and buttocks against the back of the chair). Do NOT have them leaning over. Subject should lift their feet slightly off the floor. The force pad should be on the side of the "best" arm (subjects will decide which arm is their strongest and healthiest prior to testing but use same arm throughout testing). Adjust the chair so the palm of the "best" arm is lightly touching the force pad for the arm to be 90 degrees at the elbow joint. The arm should not be tense, it should be relaxed. The opposite hand will be holding the side of the chair. Administrator will say Ready, Set, Go. When the administrator says Go, subject is to push as hard and as fast as they can until the administrator says Relax. Do NOT use the body to generate more force. Make sure you are not pressing the force pad before administrator says Go and that you do not "jerk" at the start of the test. The subject will perform 3 trials with the best arm. Try and produce maximal efforts on each trial.

Testing Protocol and Procedures:

- Subjects will perform at least 3 trials with the "best" arm for 6 sec.
- If the third trial force production is greater than the previous two trials then perform another trial until a lower value is achieved. Perform a maximum of 5 trials. If the 3rd trial force production is lower than the previous two trials, then the test is completed.
- Have subjects hold the isometric muscle action for the specified time (6 sec.)
- Allow 30-60 secs. between individual trials.

GRIP STRENGTH PROTOCOL

1. Subject is standing, feet shoulder width apart, staring straight ahead.

2. Subject's head should be in the midposition (facing straight ahead)

3. Subject's elbow may be flexed anywhere between 90 and 180 degrees, with hand and forearm in mid-prone position. (see Figure)

4. The grip size should be adjusted so that the middle finger's midportion (second phalanx) is approximately at a right angle.
   a. Grip adjustment #1 = the slot at the innermost position for the smallest grip size (see Figure)

   b. Grip adjustment #2= the slot at the outside position for the largest grip size

5. The subject should squeeze quickly and maximally for three seconds.

6. The subject should make three trials alternately with each hand, with at least 30 s between trials for the same hand.

The tester should record each maximal value in kg and then multiply by 10 for Newtons The tester should circle the best maximum score for each hand.

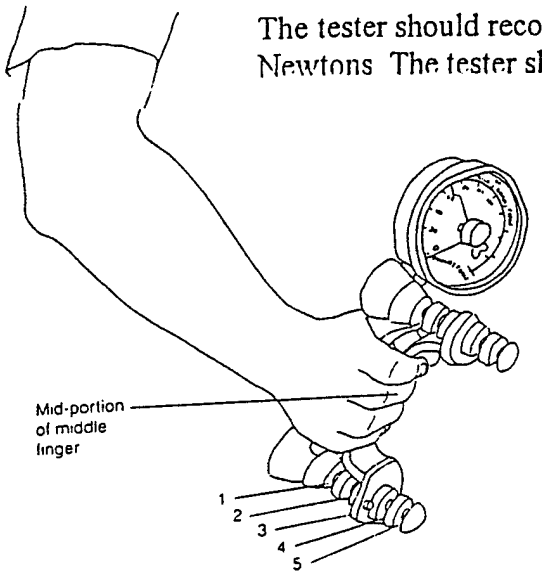


Figure 2 The proper positioning of the body, upper arm, forearm, and hand during handgrip strength testing.

1. *Warm-up* 5 to 10 min of stretching and a few vertical jump at 50 to 70 % effort.
2. *Standing Reach*: The subject will stand with the feet together and her preferred side against the wall. Then she will raise her preferred arm as high as possible so that the palm of the hand is against the wall.
3. *Jumping Reach*: Chalk will be put on the middle finger of the preferred hand and the subject will move to a jumping position. Vertical jump will be performed from a two footed standing position with a counter movement (one quick dip of the knees). Subjects will be allowed to use their arms (swing of the arms). But, they must jump without a step into the jump.

BICEP CURL 1RM PROTOCOL

1. TEST SPECIFICS
    a. All testing is performed using a dumb bell.
    b. Warm-up
    c. Bicep Curl 1RM Protocol
        1. Spotter is needed to stand in front of subject for subject's safety and for a missed 1RM attempt.
        2. Subject assumes correct testing position, standing in an anatomical position facing towards administrator with piece of paper being supported between lower back and wall, feet shoulder width apart, knees slightly flexed, "best" arm (subject decides which arm is the most healthy but use same arm throughout testing) with palm facing forward and other arm with hand resting on hip.
        3. Subject is given dumb bell in "best" hand.

4. Test begins when subject begins to lift arm with dumb bell upward until dumb bell touches front of shoulder and then lowering it to the starting point, completing the full range of motion.

d. Contraindications to a successful attempt
   1. Subject fails to touch dumb bell to shoulder and then return to original starting position.
   2. If subject does not maintain the proper position during attempt (i.e. hips or back move from the wall causing the piece of paper to fall to the ground) the attempt will be failed by the administrator.
   3. Spotters willingly or inadvertently make contact with dumb bell during the lift.
   4. Subject fails to touch shoulder with dumb bell during lift.

e. Administration of attempts
   1. It is the goal of the test supervisor to attain a 1RM within three attempts.
   2. It is the task of the supervisor to determine the load increase after a successful attempt, using visual inspection judgment, and vocal feedback from the subject.
   3. If the subject fails at an attempt that is five or more kilograms greater than the previous successful attempt, the subject is granted a final attempt at a reduced weight-appropriately between the successful and unsuccessful attempts.

Standard Operating Procedure - KinCom

Turn on KinCom, 30 sec. later turn on computer
Get subject on the KinCom machine
Place lever arm box at right ankle level and record level
Strap subject onto machine with ankle strap, thigh strap and waist strap
Enter subject identification info into the computer
Measure new gravity and position parameters
Set parameters for lever arm motion
Instruct subject to sit up straight (back not touching chair back) and cross arms across their chest
Have subject extend leg as forcefully as possible through the full range of motion, then flex leg back to the starting position as forcefully as possible
Perform a total of 3 sets at each speed.
Speeds used are 30 °/s, 90 °/s, and 180 °/s.
Save info on computer
Unstrap subject and help off the KinCom machine

General Clinical Research Center

Skin Folds

Subject : _____ I.D.No. 5 Date 5/11/2001

Weight: 63.6 Kg 140.2 lbs

Height: 163.4 Cm 64.3 In

Age 58 Technician _____

Sex F Study : _____

Skinfold Measurements

Skinfold Sites	1st Test	2nd Test	3rd Test	Average
Triceps	29.0	29.0	29.0	29.0
Subscapular	23.0	25.0	28.0	25.3
Chest	9.0	8.0	8.0	8.3
Midaxillay	25.0	26.0	25.0	25.3
Suprailiac	17.0	14.0	15.0	15.3
Abdomen	24.0	24.0	22.0	23.3
Thigh	38.0	39.0	40.0	39.0
Circumferences				
Waist	78.0	78.0	78.0	78.0
Hip	94.0	94.0	94.0	94.0

Results

Waist/Hip Ratio	% fat Men	% fat Women
0.83	25.94	31.93

Exercise Protocol

Note: Participant should not be exercising if most recent meal was more than 3 hours ago. A light snack 40 minutes to 1 hour before exercise is recommended, and having a water bottle during the session is strongly encouraged.

1. Warm-up on the recumbent bike or on the treadmill at a steady, pace for 5 minutes
2. Light stretching for 5 minutes
3. Resistance exercise protocol:
    - **Week 1**: one set of each exercise – 60 second rest breaks between exercises – 10 to 12 reps
    - **Week 2**: two sets of each exercise – 60 second rest breaks between each set and between exercises – 8 to 12 reps
    - **Week 3 – 24**: three sets of each exercise – 90 second rest breaks after squats, 60 seconds for rest of exercises – 8 to 12 reps

*** We are emphasizing the rest break be no longer than 60 seconds (90 seconds following squats) in order to maintain a continuous, quality workout**

Exercises:

Squats:

Feet shoulder width apart
Hold weights in front of body
Keeping back straight and head up
Lean slightly forward to achieve full range of motion
Squat down and come up quickly keeping arms extended and weights down

Leg extensions:

Subject lies face down
Figure 8 wrap the Velcro strip around the ankle
Subject holds band with both hands at the base of the neck
Subject may gather up more band in order to increase resistance
Beginning with knee at a 90-degree angle, lower the leg to ground and raise up
Get the full range of motion in the knee

Record band color and amount of band gathered in the hand

Leg curls:

Protocol 1:

Subject lies face down
Band horizontally over heel
Trainer holds band, maintaining constant resistance, while subject moves knee through the full range of motion

Record band color

Protocol 2:

Subject lies face down
Trainer stands above subject, pressing down on ankle
Subject moves knee through the full range of motion

* Choose one protocol or the other. Do not do both

Calf raises

Subject stands with front half of foot on a platform
Lower heels in a controlled manner, and then lift them to the starting position
One or two feet at a time

Chest Press / Push-ups

Start doing push-ups against the wall, and as subject gets stronger, move feet further from wall until subject is ready to move to the floor
Keep hands shoulder width apart and back straight

Record the distance of feet from the wall and number of reps

Bicep Curl

Feet shoulder width apart
Grasp one weight in each hand in front of the body
Curl arms upward
Return to start position slowly
Keep elbows close to the body

Record weight and number of reps

Tricep Press-Down

Wrap one band horizontally around leg of table, chair, treadmill etc.
Thread the other band through the loops on the first band, giving you two "handles"
Subject lies on back, with head closest to table, chair, treadmill, etc.
Start with some tension in band
To increase or decrease resistance, move further or closer to treadmill
Keeping elbows on the ground, lower hands towards hips
Full range of motion
Return to start position slowly

Record band color and distance of top of head from treadmill/chair/table/etc.

Row

Place left knee up on bench
Stabilize with extended left arm
Keep right foot flat on the floor and leg straight
Right arm is fully extended toward ground
Pull straight up
Return to start position slowly
Keep back straight

Record weight and number of reps

Upright Row

Feet shoulder width apart
One weight in each hand, palms facing body
Pull weights up to chin
Return to start position slowly
Keep back straight
Weights should be parallel to floor at all times

Record weight and number of reps

Crunches

Two sets of 10-20 crunches depending on the subject
One style: lying on back, place feet on a chair with knees bent at 90 degree angle. Place hands on thigh and "crunch" torso up, while sliding hands up thigh towards knee.

3. Aerobic Exercise

(Note: Maximal heart rate is estimated by subtracting your age from 220.)

Weeks 1 and 2: 15 minutes
Maintain heart rate at approximately 60% of maximal heart rate
Weeks 3 and 4: 20 minutes
Maintain heart rate between 60-70% of maximal heart rate
Weeks 5-24: 20 minutes
Maintain heart rate around 75% of maximal heart rate

*During weeks 5-24 add 2 minutes per week onto the aerobic exercise segment.

4. 5 minute cool down, quiet walking and stretching

Subject Initials _____ Date:_____ Witness Initials:_____ Date:_____

INFORMED CONSENT FORM

Dept. of Biochemistry and Molecular Biology/Dept. of Kinesiology

The Pennsylvania State University

Study Title: "The Use of Exercise to Increase CD4+ T Lymphocytes Following Chemotherapy Treatment for Breast Cancer"

Investigators: Andrea Mastro, Ph.D.; Nancy I. Williams, Sc.D.; William J. Kraemer, Ph.D; Richard H. Dixon, M.D.; Aaron D. Bleznak, M.D.; and Judy Underwood, R.N. and Elizabeth Orsega-Smith, Michael Perry, Kate Wagner

Subject Name:_____ **Investigator:**_____

Description of the Study, Test Profile, and Associated Risks and Benefits for the Subject

Background for the Study

The causes of breast cancer remain unknown. It has been observed that following chemotherapy certain white blood cell (called CD4+ T lymphocyte) levels are significantly reduced. These cells are very important in the response of the body to fight foreign agents such as bacteria, viruses, and tumor cells. It has also been observed that no drugs can successfully increase this cell count. The mechanisms by which these white blood cells are increased in the body is not well understood. We feel that some type of exercise therapy program may help to increase these cells. If exercise does help, then such therapy can be used to enhance the health of women. In this study we propose to determine the influence of exercise training and its impact on these white blood cell levels. In addition, we want to see how exercise affects your functional abilities following chemotherapy. After preliminary testing, you will be randomly assigned ("meaning like the toss of a coin") to one of two groups. Subjects in the study will go through a familiarization period prior to testing and training (exercise groups) so that you know how to perform the tests and exercises if you are in one of the training programs.

How the Study Will Be Set Up.

To be in the study you must have a specific low cell count of a specific white blood cell (CD4+ lymphocytes of 500 per microliter or less). You must be able to perform normal daily activities and be approved by your physician based upon your medical background. If you initially qualify based upon your specific white blood cell count, you will then be asked to participate in the exercise portion of the study and undergo further testing and evaluations. We would then randomly place you (your group would be determined by chance) into one of the two groups. You would either be placed into a mild exercise (ME) or light exercise (LE) group. The exercise program will consist of a three month class consisting of exercise sessions three times per week. You will then be encouraged to continue to exercise at home for an additional 3 month period. The exercise class will last for 3 months.

After the 3 month class, exercise will continue in a home-based program for an additional 3 months. During the home-based program, exercise logs and a visit to the class 1 time every 2 weeks will allow us monitor your further progress. Testing will take place at pretraining, 3 months, end of exercise and during a follow-up period.

Testing Profile and Associated Risks

Blood Samples.

We will ask to obtain a blood sample to determine if you have a low cell count (500 CD4+ white blood cells or less in a microliter of blood). If you qualify for the study we will then request blood samples at 0, 3 and 6 months of the program (including control subjects). The blood will be analyzed for all of the different types of white blood cells. This will tell us how your immune system is responding. To obtain the blood we will use a syringe and needle and make a puncture into one of your arm veins. We will take the blood between 7 and 10 am in the morning after an overnight fast. We will ask that you perform no exercise for 48 hours before the blood test. We will take about 20 ml (4 teaspoons) of blood. A pint of blood is the amount of blood typically obtained when you donate blood. Blood sampling in this experiment will not affect your health or physical capabilities. With the blood sampling, the risks to you are of local discomfort and pain, passing out, and black and blue bruises on your skin. Stationary clots, a moving clot lodging in a blood vessel, and infections are potential risks, but are of very rare occurrence. These risks can be reduced or eliminated by having trained and proficient investigators using aseptic techniques and having additional assistants closely monitor you while your blood is being obtained in a seated position. All blood samples will be drawn in the laboratory or clinic under sterile conditions.

Questionnaires:

We will ask you to complete the following questionnaires at each blood draw time point once you are enrolled in the study. Instructions as to how to fill in the forms will be provided if needed.

- **Quality of Life Questionnaire:** The so called "quality of life" questionnaire asks questions about how you feel. You can choose to answer only the questions you want or none of them.
- **Diet Questionnaire:** You check off all of the food, beverages, supplements that you consumed in a week before you begin/began chemotherapy, when you have completed chemotherapy, when you have completed radiation therapy/before you start the exercise program, after three months of exercise and after six months of exercise.
- **Physical Activity Questionnaire:** This form asks questions about your physical activity over a week.
- **Self-perception questionnaires:** These questionnaires assess your self-perception. If the questions make you uncomfortable, you may choose not to answer them.

Body Composition Assessment/Anthropometric Measures.

We will obtain your body weight and height using a physician's scale with height measure. We will also take various arm, waist, and leg circumferences using a tape measure. We will also obtain skinfolds with a caliper which measures the amount of fat you have by taking a "skinfold" or pinch of your skin and fat at various anatomical sites on the front and back of your arms, upper thigh, upper back, side of your lower leg, and stomach. We will use a skinfold caliper which measures the thickness of the fat fold between two contact tips that apply pressure to each side of the fold. Such data will allow us to determine your % body fat. You may feel a slight pinch or squeeze on the skinfold sites from the caliper, but no other known risks are associated with the skinfold measurements.

Cardiovascular Testing and Training:

A graded exercise test on an exercise cycle will be used to assess endurance fitness (called "peak oxygen consumption"). This test will start at a level you can easily perform and progress to harder and harder levels of work every 2 minutes. You will be asked to continue the test until you feel that you can no longer do the exercise. We also can stop the test at any time if we feel that you are not able to continue. This test will serve as a test of your heart's ability to exercise and tell us about your endurance. During the test we will be monitoring your heart rate by attaching electrodes to your chest and hooking up an EKG machine. This machine will pick up the electrical signals from your heartbeat for us to look at and monitor the test. We will also ask you to rate your feeling of fatigue from a numbered scale ranging from very easy to very hard. We will also ask you to breathe into a mouth-piece (like a scuba diver) and we will analyze how much oxygen and carbon dioxide is in your expired air in order to measure the amount of oxygen you consume. We will also measure your blood pressure in two-minute stages by putting a blood pressure cuff around your arm, pumping it up and listening to your heart by using a stethoscope placed on your arm. You will be the one who decides how long you can ride the bike and can stop at any time you want. During exercise testing and with training, you might experience episodes of lightheartedness, chest discomfort, and/or leg cramping. Occasional irregular heart beats and/or abnormal blood pressure responses and chest discomfort are also possible. Other potential risks include the possibility of muscle strain or pull, muscle soreness, fainting, and nausea. We will attempt to minimize this possibility by familiarizing you with the procedures, and by allowing you to practice and warm up prior to the tests and training sessions. The risk of heart attack, although very rare, (approximately 1 occurrence per 15,000 tests), does exist. Every attempt will be made to eliminate such risks through the use of medical screening, the use of trained and experienced personnel monitoring the test and training sessions, established emergency precautions and procedures, and proper testing and training procedures employed in the investigation. All personnel involved with the testing are currently certified in CPR. Access to further medical care will be provided to you in case of emergency.

Muscle Function Testing and Resistance Exercise Training:

In order to determine the changes in your ability to use your muscles we want to test you in a number of strength and power tests of muscle function. We will use a hand grip strength test to see how hard you can squeeze your hand. You will be given three attempts and we will use the highest score. We also want to see how much power you can produce in a vertical jump. We will measure this by having you stand on a special plate built into the floor and ask you to jump as high as you can with your hands held on your waist. We will ask you to perform a jump three times with about 2 minutes rest between your jumps. Again we will use the highest jump for analysis. We will ask you to perform a strength test on a strength testing machine (called an Biodex isokinetic tester) in the seated position. We will ask you to kick out your leg and pull it back as fast as you can three times with rest between attempts. We will ask you to do this at three different resistances. During testing, you will be stabilized by thigh, chest, and waist straps. This test will give us a measure of your physical strength at three different speeds of movement. We will also ask you to perform several bicep curls with dumbbells, and to press hard on a pad for several seconds to assess your arm strength and endurance. The performance of muscular exercise and physical effort can entail a certain degree of hazard for injury from overexertion and/or accident. This study will be planned to avoid injury to the muscles and

bones. Every effort will be made to make this investigation safe for your participation through familiarization (teach you how to perform the tests), experienced personnel, warm-up and cool down (i.e., stretching and low intensity activity specific exercise), technique instruction and practice, supervision, screening, and monitoring while testing. Strength testing incorporates the risk of pulling or straining or tearing a muscle, and increases your blood pressure. The proper use of testing devices decreases the risk of dropping the weight, as does investigator observation and assistance. In order to decrease the risk of injury, you will asked to warm up with a light weight and rest between attempts. Proper technique will be taught and enforced. To prevent dizziness and attenuate increases in blood pressure associated with the strength maneuver, you will asked not to hold your breath and to relax between efforts. Some post-exercise muscle soreness may be experienced 24 to 72 hours after the testing or training session. Such symptoms should disappear completely within a few days and have no residual effect. These risks can be reduced or eliminated by close supervision during the testing so that proper form and no jerking movements during a movement are utilized and by having you properly positioned for each specific test. The risks are similar for training, but with the use of supervised and progressive programs starting at levels you can tolerate, any risks related to training are rare. The risk to you will also be reduced by having experienced personnel conduct each test and training session.

Exercise Training Programs.

Each exercise training session will last approximately 1 to 1.5 hours. Exercise programs for the ME and LE exercise groups have been designed to safely accommodate training in women of all ages. The program will be individualized for you and you will only be asked to do what you are capable of doing. The LE group will perform movement exercises, stretching exercises, yoga, relaxation exercises and light resistance exercise. The ME group will perform moderate aerobic exercise and moderate resistance exercise training. You will be supervised for the first 3 months by coming to three class periods a week. You will be asked to continue your exercises at home for three more months. While exercising at home, you will be asked to keep an exercise log. You will be provided information about this log before you begin exercising at home. During this time, we will ask you to come into class once every two weeks to see how things are going, collect your exercise logs, and answer any questions you might have.

Benefits to the Individual Subjects who Participate in the Study.

The benefits to *YOU* in this study will come from learning how to exercise and the extensive testing information provided to you free of charge. You will also gain information on your immune system's response and on your exercise performance abilities. All tests will be explained and interpreted for each subject and questions will be answered so that a maximum amount of educational understanding and use of the data will be achieved. We will also send copies of your results to your physician.

Statement of confidentiality:

All records associated with my participation in the study will be subject to the usual confidentiality standards applicable to medical records (e.g., such as records maintained by physicians, hospitals, etc.) and in the event of any publication resulting from the research no personally identifiable information will be disclosed.

I,_____, having the full capacity to consent, do hereby volunteer to participate in a research study entitled, "The Use of Exercise to Increase CD4+ T Lymphocytes Following Chemotherapy Treatment for Breast Cancer" under the primary direction of Andrea Mastro- Ph.D. and Nancy I. Williams, Sc.D. of the Department of Biochemistry and Molecular Biology and the Department of Kinesiology. The implications of my voluntary participation; the nature, duration and purpose; the methods and means by which the study is to be conducted; any inconveniences and hazards which may reasonably be expected have been explained to me by _____and are set forth on the prior pages of this agreement, which I have signed.

I have been given an opportunity to ask whatever questions I may have had and all such questions and inquiries have been answered to my satisfaction. Should any further questions arise, I will be able to contact: Dr. Mastro at 863-0152 or Dr. Nancy I. Williams at 865-1346 or, for medical questions, Dr. Dixon at 234-8800, or Dr. Bleznak at 231-4560. I understand that I am free to ask questions concerning my participation at any time during my participation in the study. I further understand that any data will remain confidential with regard to my specific identity. It should be noted that representatives of the U.S. Army Medical Research and Material Command are eligible to review research records as a part of their responsibility to protect human subjects in research.

I will be authorized all necessary medical care for injury or disease which is the proximate result of my participation in this research. The U.S. Army requires that this institution provide such medical care when conducting research with private citizens. Other than the medical care that may be provided, I understand that I will not receive any compensation for injury from my participation in this research study, however I understand that this is not a waiver or release of my legal rights.

I FURTHER UNDERSTAND THAT I AM FREE TO WITHDRAW MY CONSENT AND TERMINATE MY PARTICIPATION AT ANY TIME WITHOUT PREJUDICE!

Date	**Date of Birth**	**Subject's Printed Name**
Permanent Address		**Subject's Signature**

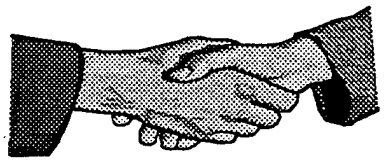
I, the undersigned, have defined and fully explained the investigation to the above subject.

Date	**Signature of Investigator**

I was present when the study was explained to the subject(s) in detail and to the best of my knowledge and belief it was understood.

Date	**Signature of Witness**

Do You Have Breast Cancer or Know Someone Who Does?



We are conducting a study examining the relationship between immune function, quality of life, and physical activity in women who have breast cancer.

To Qualify You Must:

- **Be Able To Carry Out Daily Activities**
- **Be Diagnosed with Breast Cancer**
- **Have M.D. Consent**
- **Be 25-80 Years Old**

Benefits To You:

- **Information On Your Immune System**
- **Free Fitness Evaluation**

For more information please call Dr. Andrea Mastro at (814) 863-0152 or (814) 863-2434.

Exercise and the Recovery of CD+4 Lymphocytes Following Chemotherapy for Breast Cancer

Participant Summary Report

This report summaries the measurements collected from you at several time points during the study which could include before chemotherapy, before exercise training began, and approximately three and six months later. It provides information to help you understand and interpret the results. Keep in mind that the exercise testing was at done at specific times. When evaluating the effects of exercise, your progress over time and your own feelings of accomplishment are important factors.

Background

Breast cancer patients, as a consequence of the disease and chemotherapy, have depressed immune systems - abnormally low blood levels of certain types of lymphocytes. In normal healthy females the number of immune cells increased after three months of exercise training including specific types of resistance exercise. This study will help determine if similar exercise training can help increase the number of these immune cells in breast cancer patients after chemotherapy. In addition, study participants should experience a decrease in fatigue and an increase in physical fitness and feelings of well being.

During your participation in the study, data was collected at several points in time about your **Blood Content, Physical Fitness** and **Body Composition.** This report contains information about each of these measures, your results and norms or ranges for comparison. Note that norm tables vary by source and only provide a general idea of average values.

Blood Content

White blood cells, which include lymphocytes, help your body protect itself against infection. This study was concerned with one type of white blood cell, the lymphocyte, which is responsible for acquired immunity - resistance that results from exposure to a foreign stimulus. There are several classes of lymphocytes. This study looked specifically at:

CD4+T cells – T helper lymphocytes; immune regulators

CD8+T cells – T cytotoxic lymphocytes; kill virally infected cells and tumor cells

B cells – B lymphocytes; make antibodies

NK cells – Natural killer cells; kill virally infected cells and tumor cells

Your blood samples were analyzed to determine percentages and total numbers of these four lymphocyte classes and the ability of these lymphocytes to respond to the presence of foreign stimuli. We used several types of plant proteins (PHA, ConA and PWM) to which the human body reacts similarly as it does to bacteria or viruses.

Normal Healthy Adult Levels	Number of Cells per Microliter of Blood	Percentage of Total Cells
White Blood Cells	4600 – 7100	
Lymphocytes	1600 – 2400	28 - 39% of white blood cells
CD4+ T cells	700 – 1100	38 - 46% of lymphocytes
CD8+ T cells	500 – 900	31 - 40% of lymphocytes
B cells	200 – 400	11 - 16% of lymphocytes
NK cells	200 – 400	11 - 16% of lymphocytes

Physical Fitness

Indicators of physical fitness include cardiovascular measures such as blood pressure, resting and maximum heart rate (pulse), cardiorespiratory fitness and muscular strength.

Blood Pressure, the force of blood flowing against artery walls, has a high point when the heart contracts called systolic pressure and a low point when the heart relaxes called diastolic pressure. It is measures in millimeters of mercury – mm Hg.

	Classification	Range
Systolic	Normal	less than 140
	Borderline Hypertension	140 to 159
	Isolated Hypertension	greater than 159
Diastolic	Normal	less than 90
	Mild Hypertension	90 to 104
	Moderate Hypertension	105 to 114
	Severe Hypertension	greater than 114

Heart Rate or pulse is the number of heart beats per minute (bpm). Average normal resting heart rate ranges from 60 to 80 bpm. Maximum heart rate is the fastest and hardest your heart muscle can beat (contract) and roughly equals 220 minus your age. Maximums drop slowly as we age.
Maximal Allowable Training Heart Rate (MATHR) is the maximum heart rate that should not be exceeded during an exercise session. It should be between 50 to 80 % of your maximum rate. For example, a 16 year old's Maximum Heart Rate is 204 (220-16) and Maximal Allowable Training Heart Rate is between 102 and 163 (204X50% and 204X80%)

Cardiorespiratory or Aerobic Fitness relates to the ability of the circulatory and respiratory systems to supply oxygen to the muscles and the ability of the muscles to extract the oxygen during sustained physical activity. Oxygen is used for the combustion of fat and carbohydrates creating the energy necessary to perform the work.

Vo2 max is a measure of the body's maximal oxygen uptake which indicates aerobic fitness. Your aerobic fitness was tested on the stationary bicycle when you continuously cycled with increased resistance. The oxygen used during the maximal effort is the maximal oxygen uptake (Vo2 max). Higher Vo2 max indicates better fitness.

Absolute Vo2 max is the maximal oxygen uptake expressed in liters per minute. Absolute Vo2 max is important in activities where body weight is not lifted such as in cycling, swimming and handling of objects and weights, other than body weight.
Relative Vo2 max is absolute Vo2 max divided by body weight and is expressed in milliliters of oxygen per body weight (kg) per minute (ml/kg/min). Relative Vo2 max is important in activities where body weight is lifted such as in walking, running and climbing uphill.
In adults, relative Vo2 max is more important than absolute Vo2 max as a health indicator.

Relative VO2 Max Norms for Healthy Females - Range by Age

Classification	<30 years	30-39	40-49	50-59	60-69
Poor	<24	<20	<17	<15	<13
Fair	24-30	20-27	17-23	15-20	13-17
Average	31-37	28-32	24-30	21-27	18-23
Good	38-48	34-44	31-41	28-37	24-34
Excellent	>48	>44	>41	>37	>34

Muscle Strength is the ability of the neuromuscular system to produce force. Some of the measures of muscle strength we used were controlled lifting of dead weight (barbells) and force against resistance (for example grip strength dynamometer for the arm muscles and the KinCom machine for the leg muscles). Tricep and Bicep strength measured in pounds was tested in one arm. Vertical jump height is measured in centimeters.
Grip Strength or force using the hand-grip dynamometer is measured in kilograms.

Grip Strength Female Norms In Kilograms for Combined Right and Left Hand Range by Age

Classification	30-39 years	40-49	50-59	60-69
Poor	<55	<54	<50	<47
Fair	55 - 60	54 - 58	51 - 54	47 - 50
Average	61 - 65	59 - 64	55 - 58	51 - 53
Good	66 - 72	65 - 71	59 - 64	54 - 59
Excellent	>72	>71	>64	>59

Body Composition

Body Mass Index (BMI) correlates relatively well with the body's content of fat, and is used as a predictor of obesity. However, BMI does not take into consideration whether a person's weight is due to fat or muscles. BMI is easy to calculate BMI = weight (kg) / height $(m)^2$.

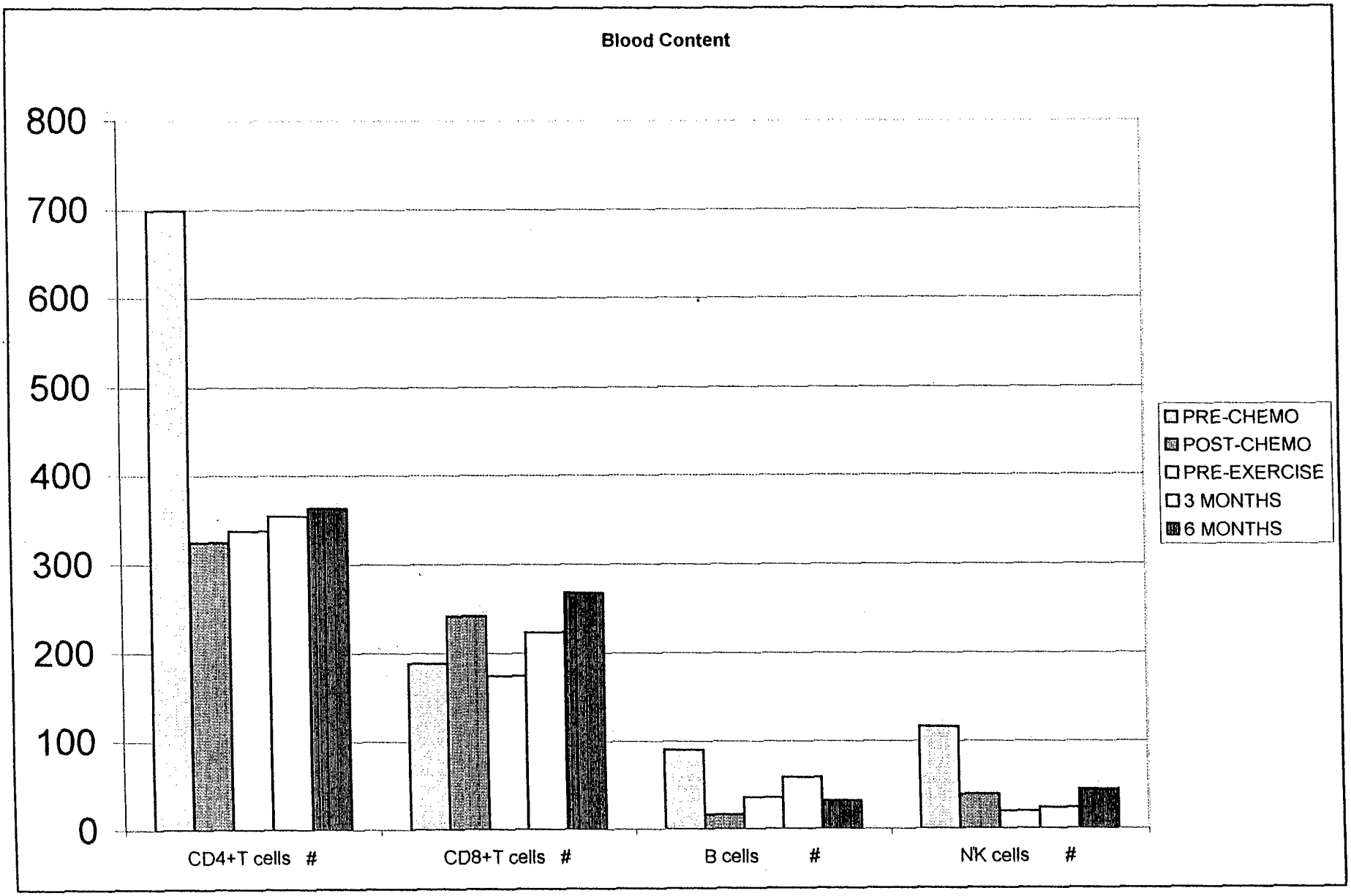
Classification	Range (According to the World Health Organization)
Normal	18.5 to 24.9
Overweight	25 to 29.9
Mildly Obese	30 to 34.9
Moderately Obese	35 to 39.9
Extremely Obese	40 and higher

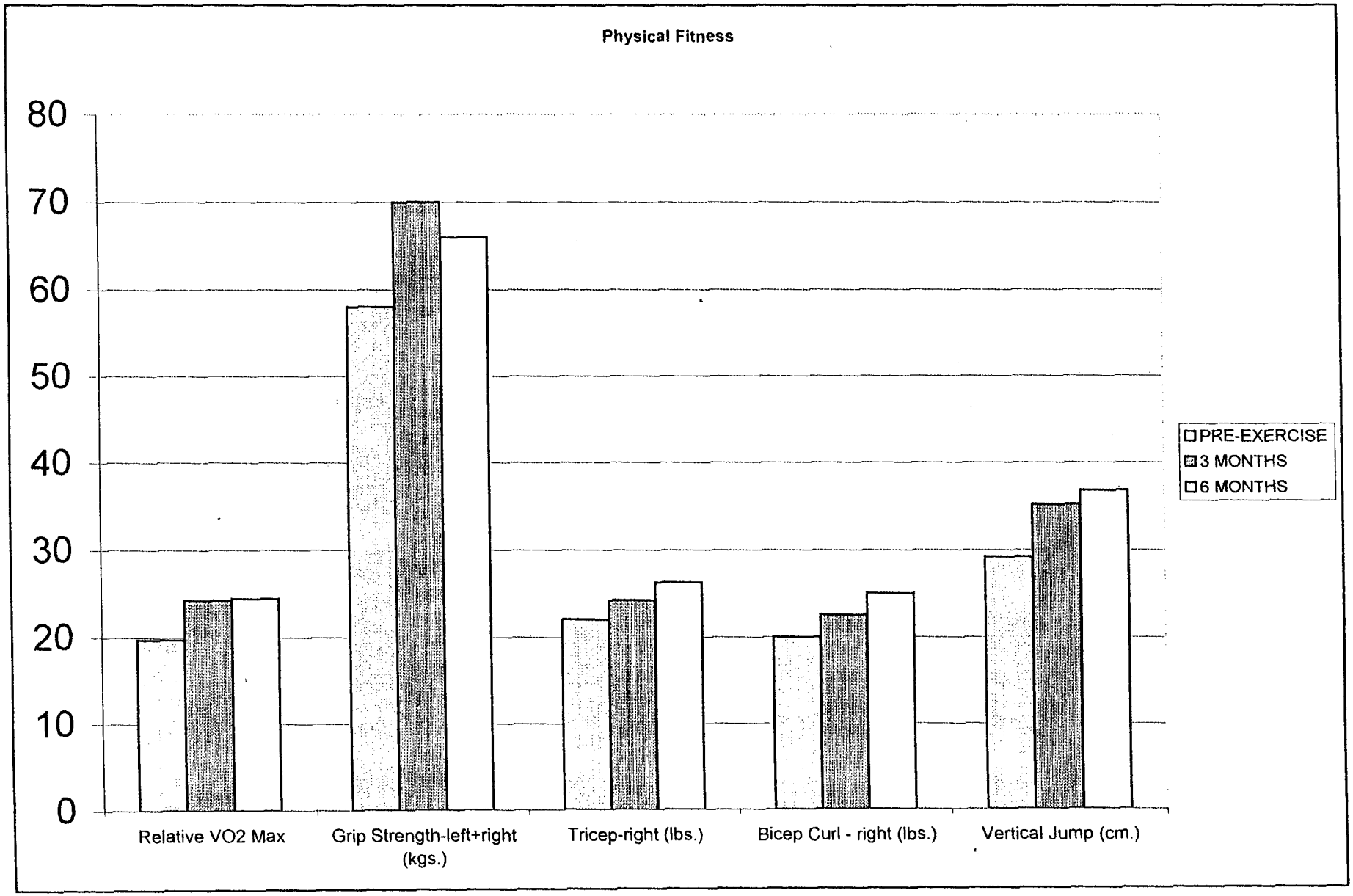
Body Fat Percentage is the relative amount of fat that you are carrying around in everyday life. Body fat percent was calculated based on your skinfold measures which are not exact and can vary based on technician, instrument, and time of day.

Classification	Range
Lean	< 20%
Normal	20 to 26%
Moderately Over Weight	27 to 31%
Extremely Over Weight	32 to 36%
Obese	greater than 36%

Waist to Hip Ratio (WHR) is a simple ratio which indicates body fat distribution more accurately than body mass index. The WHR for a woman with a 26 inch waist and 36 inch hips would be 0.72 (26/36). Normal/average is 0.80. A woman's waist-to-hip ratio should be less than 0.85. Greater than 0.88 indicates excessive abdominal fat and is associated with increased health risks.

Exercise & Breast Cancer Participant #4100MS Report					
	PRE-CHEMO	POST-CHEMO	PRE-EXERCISE	3 MONTHS	6 MONTHS
BLOOD CONTENT					
White blood cells #	6200	2000	3400	3300	4000
Lymphocytes #	1400	650	600	700	800
CD4+T cells #	699.16	325.26	338	355	363.4
CD8+T cells #	188	242	174	223.4	268.24
B cells #	90.16	16.25	35.76	58.87	32.16
NK cells #	116	39.26	19.8	24	44.64
Lymphocytes %	23.2	32.4	17.6	22.2	20.3
CD4+T cells %	49.94	50.0	56.4	50.7	45.4
CD8+T cells %	13.44	37.2	28.9	31.9	22.1
B cells %	6.44	2.5	6.0	8.4	4.0
NK cells %	8.26	6.0	3.3	3.4	5.6
Response to Foreign Stimuli					
PHA - Maximum	21481	23624	13983	53705	28993
ConA - Maximum	3280	10168	4526	13763	7153
PWM - Maximum	1481	3869	2286	7130	5298
PHYSICAL FITNESS					
Blood Pressure - Resting			130/74	110/68	128/78
Heart Rate - Resting			72	72	68
Heart Rate - Exercise Max			157	173	166
Relative VO2 Max			19.7	24.2	24.4
Grip Strength-left+right (kgs.)			58	70	66
Tricep-right (lbs.)			22	24.2	26.3
Bicep Curl - right (lbs.)			20	22.5	25
Vertical Jump (cm.)			29.2	35.2	36.8
Thigh/Hamstring low resistance			74/40	82/43	99/60
Thigh/Hamstring medium resistance			69/69	93/47	83/53
Thigh/Hamstring high resistance			87/30	91/40	74/46
BODY COMPOSITION					
Body Mass Index			28.01	28.76	28.84
Body Fat Percentage			39.97	40.45	40.32
Waist to Hip ratio				0.95	0.84


Blood Content
800
700
600
500
400
300
200
100
0
CD4+T cells #
CD8+T cells #
B cells #
NK cells #
PRE-CHEMO
POST-CHEMO
PRE-EXERCISE
3 MONTHS
6 MONTHS

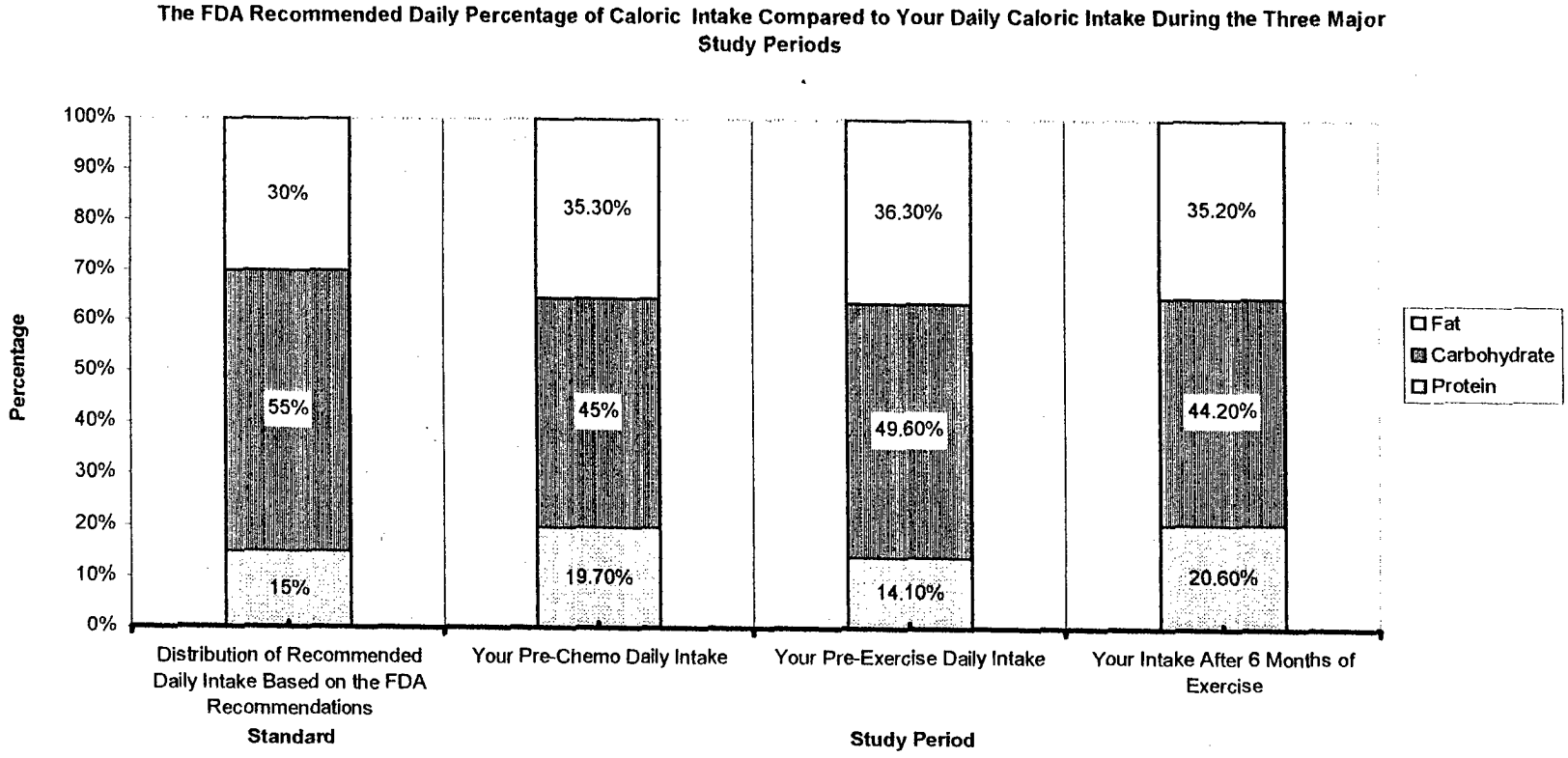

Physical Fitness
80
70
60
50
40
30
20
10
0
Relative VO2 Max
Grip Strength-left+right (kgs.)
Tricep-right (lbs.)
Bicep Curl - right (lbs.)
Vertical Jump (cm.)
PRE-EXERCISE
3 MONTHS
6 MONTHS

Client Nutrient Analysis

BC5201BV

Nutritional Summary:

All nutrient values are compared to Food and Drug Administration (FDA) Recommendations based on a 2000 Calorie Adult Diet



- Please note that your vitamin and mineral intakes seen below do not take into account any supplements that you may take such as multivitamins or other various supplements.

*= No Recommended Daily Allowance (RDA) Goal Established by the FDA

Macronutrients

Nutrient	Your Intake Pre-Chemo	% Of RDA Goal	Your Intake Pre-Exercise	% Of RDA Goal	Your Intake 6 Months of Exercise	% Of RDA Goal
Calories	3106 Cal	155 %	1700 Cal	85 %	1725 Cal	86 %
Cholesterol	456 mg	152 %	141 mg	47 %	298 mg	99 %
Saturated Fat	40.0 g	200 %	17.5 g	88 %	21.8 g	109 %
Monounsaturated Fat	47.6 g	*	23.6 g	*	27.1 g	*
Polyunsaturated Fat	24.8 g	*	24.9 g	*	12.2 g	*
Dietary Fiber	24.6 g	99 %	22.1 g	88 %	10.6 g	42 %
Sugar	157 g	*	88.9 g	*	79.8 g	*

Vitamins

Vitamin	Your Intake Pre-Chemo	% Of RDA Goal	Your Intake Pre-Exercise	% Of RDA Goal	Your Intake 6 Months of Exercise	% Of RDA Goal
Vitamin A	4641 IU	93 %	6862 IU	137 %	1789 IU	35 %
Vitamin C	163 mg	272 %	158.8 mg	265 %	100 mg	167 %
Vitamin D	3.9 µg	39 %	4.48 µg	45 %	2.01 µg	20 %
Vitamin E	19.8 mg	99 %	16.4 mg	82 %	5.16 mg	26 %
Thiamin	2.69 mg	179 %	1.64 mg	109 %	1.70 mg	113 %
Riboflavin	2.84 mg	167 %	1.94 mg	114 %	1.59 mg	94 %
Niacin	42.8 mg	214 %	21.1 mg	105 %	26.7 mg	134 %
VitaminB6	3.62 mg	181 %	2.58 mg	129 %	1.89 mg	95 %
Folate	372 µg	93 %	351 µg	88 %	140 µg	35 %
Vitamin B12	19.4 µg	323 %	11.8 µg	196 %	5.49 µg	91 %
Biotin	11.9 µg	4 %	20.1 µg	7 %	9.23 µg	3 %
Pantothenic Acid	7.21 mg	72 %	4.06 mg	41 %	3.95 mg	40 %
Vitamin K	20.2 µg	25 %	45.3 µg	57 %	12.1 µg	15 %

Minerals

Mineral	Your Intake Pre-Chemo	% Of RDA Goal	Your Intake Pre-Exercise	% Of RDA Goal	Your Intake 6 Months of Exercise	% Of RDA Goal
Sodium	3133 mg	131 %	2316 mg	96 %	mg	%
Potassium	4674 mg	134 %	3060 mg	87 %	mg	%
Calcium	896 mg	90 %	690 mg	69 %	mg	%
Iron	28.8 mg	160 %	20.9 mg	116 %	mg	%
Phosphorus	2171 mg	217 %	1105 mg	111 %	mg	%
Magnesium	454 mg	113 %	305 mg	76 %	mg	%
Zinc	25.8 mg	172 %	12.0 mg	80 %	mg	%
Copper	2.20 mg	110 %	1.37 mg	68 %	mg	%
Manganese	4.50 mg	225 %	3.72 mg	186 %	mg	%
Selenium	84.5 µg	121 %	47.4 µg	68 %	µg	%
Chromium	0.038 mg	32 %	0.023 mg	19 %	mg	%
Molybdenum	22.2 µg	30 %	19.4 µg	26 %	µg	%

Over the course of the study your macronutrient intake of carbohydrate, fat and protein remained fairly constant. Your intake of carbohydrates was below the average, so this may a nutrient that you could increase your consumption of. You also might want to take into account that for some of the Vitamins for which your intake was over 100%, your true intake may have been even higher due to supplements not included into these values. This is not a nutritional analysis so please see you dietician for your personal dietary recommendations.

Appendix #9 PERSONNEL

Because of the nature and length of this study personnel turnover has been extensive due to factors such as student graduation/schedule changes/relocation, change in employment, etc. Some university personnel worked on the project part-time. $ indicates payment.

UNIVERSITY PERSONNEL

Project Coordinator (s)
Andrea M Mastro
Beth Baker - Intern $
Beth Orsega Smith - Post Doctorate $
Kate Wagner $

Lab Technicians/Data Input
Keira Fuerner - $
Jennifer Ford (student, credit)
Michelle Yon $
Douglas DiGirolamo $
Marie St. Louis $

Exercise Trainers
Independent study credits were given to Kinesiology students working as personal trainers. In addition some received payment as indicated by a $.

Melanie Bombar –$	Amber Lee - $
Katie Dewar –$	James Marx - $
Sheri Foura - $	Susan Messics - $
Jaclyn Kalp - $	Mike Perry - $

Truie Benninger	Kristen Lowe
Melissa Border	Lena McKelvey
Chris Conboy	Tim Meredith
Daniel Day	Meredith Parker
Jessica Himler	Joe Stempien
Curry Johnson	Veronica Walker
Amber Lee	

Dietary Data Input and Analysis
Becky Kijanka
Marie St. Louis
Syreeta Cherry

GCRC PERSNNEL

University Park GCRC support personnel
Denise Sheffield, RN-VO2 testing, KinCom, strength testing

Janet Delfs, MS -VO2 testing, KinCom, strength testing
Paula Mulhall, RN- VO2 testing, blood draws, skin-folds
Laurie Aquilino, RN- blood draws, skin-folds, ECG
Rebecca Falsone, MT-blood draws
Anne Trout, MD-History and physical and ECG interpretation
Harold Bassett, DO-History and physical and ECG interpretation
Margot Putukian, MD-ECG interpretation
Nancy Lambert, RN-Scheduling

Hershey GCRC Staff
Cindy Hogeman RN-VO2 Max testing, Kin Com
Nancy George , LPN- skin folds, strength assessment, VO2 Max, Kin Com
Joanne Kirchmer,Medical Technologist(ASCP) -same as Nancy
Shirlynn Mottilla, RN- recruit,schedule medical history, blood draw, questionnaires
Dr. Sinoway MD-History and physical, read EKG
Chanty Webb, RN, Area Representative- recruitment
Remainder of the staff filled in when needed:
Kim Smalley, RN
Judy Kovach, RN
Sandy Gagliardi, secretary